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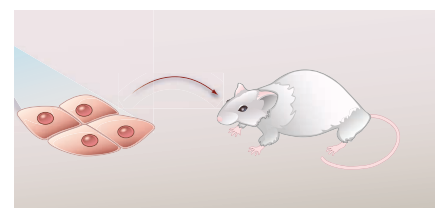
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Image: NASA Genesis Team

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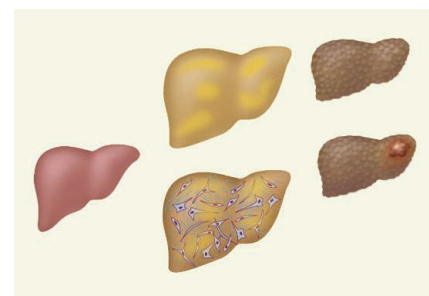
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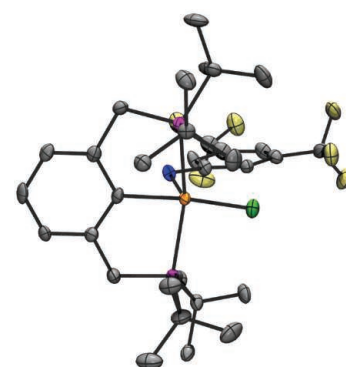
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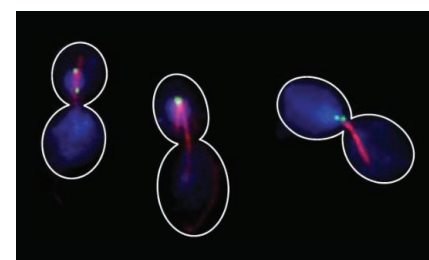
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10.1126/science.1201512

Dinosaur Body Temperatures Determined from Isotopic (^{13}C - ^{18}O) Ordering in Fossil Biominerals

R. A. Eagle et al.

Large dinosaurs had body temperatures similar to those of modern mammals and birds.

10.1126/science.1206196

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S. Hirose et al.

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10.1126/science.1203903

Organization of Intracellular Reactions with Rationally Designed RNA Assemblies

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Multidimensional RNA structures can act as scaffolds for the spatial organization of bacterial metabolism.

10.1126/science.1206938

Targeted Genome Editing Across Species Using ZFNs and TALENs

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Engineered nucleases target specific DNA sequences for gene disruption in nonmodel organisms.

10.1126/science.1207773

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Highlights From Our Daily News Coverage

Spying on a Silent Killer

A new imaging technique allows researchers to hear warning signs before a heart attack or stroke.

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Wing Hairs Turn Bats Into Aerial Aces

Research into bat flight could inspire design of new types of aircraft.

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The Iceman's Last Meal

New details emerge about diet, eye color, and cavities of a 5000-year-old traveler.

<http://scim.ag/last-meal>

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The Signal Transduction Knowledge Environment

21 June issue: <http://scim.ag/ss062111>

RESEARCH RESOURCE: Comparative Proteomic Analysis Identifies a Role for SUMO in Protein Quality Control

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PRESENTATION: HIF-1 α Mediates Tumor Hypoxia to Confer a Perpetual Mesenchymal Phenotype for Malignant Progression

Y.-G. Yoo et al.

Hypoxia-induced genetic alternations induce epithelial-mesenchymal transition and tumor progression.

JOURNAL CLUB: Partners in Crime—Ubiquitin-Mediated Degradation and Autophagy

S. M. Srinam et al.

Toll-like receptor 4 signaling provides insights into the molecular mechanism of ubiquitin-dependent selective autophagy.

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Integrating Medicine and Science

22 June issue: <http://scim.ag/stm062211>

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RESEARCH ARTICLE: Dosage Thresholds for AAV2 and AAV8 Photoreceptor Gene Therapy in Monkey

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RESEARCH ARTICLE: The Role of Nogo and the Mitochondria—Endoplasmic Reticulum Unit in Pulmonary Hypertension

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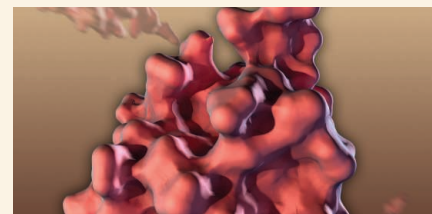
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RESEARCH ARTICLE: Immunogenicity of the Tuberculosis Vaccine MVA85A Is Reduced by Coadministration with EPI Vaccines in a Randomized Controlled Trial in Gambian Infants

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Coadministration of tuberculosis vaccine with standard childhood vaccines may reduce its immunogenicity.



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SUMO, a ubiquitin-like protein.

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V. Raper

The decision to retire has professional, financial, and psychosocial aspects that scientists need to plan for well in advance.

http://scim.ag/retirement_vraper

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A. Ruben

Our labs are science-based mini-societies—so why do we run them in the same arbitrary and bureaucratic way as the rest of the world?

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Science Policy News and Analysis

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ADVANCING SCIENCE. SERVING SOCIETY



Make Your Own Bed

Animals move through their environments to search for resources, and so movements that provide the greatest efficiency should be advantageous. As animals move and extract resources, however, the search environment is altered by their activity. Thus, search efficiency can be a moving target. **De Jager *et al.*** (p. 1551; see the Perspective by **Grünbaum**) show that individual movements in mussels generate beneficial environmental complexity at the population level. Specifically, individuals move using a type of random walk that consists of both long and short bouts, the cumulative effects of which generate a patchy distribution of mussels within the mussel bed. This patchy distribution confers a fitness advantage to individuals, and it is the walk strategy that allows for the development of this beneficial environment.

Foie Gras: For Birds Only!

The capacity to store excess fat in liver is beneficial to migrating birds in need of energy, but in humans, fatty liver is maladaptive and can have serious clinical consequences. Accompanying the trend toward unhealthy diets, nonalcoholic fatty liver disease (NAFLD) now affects about one-third of adults in developed countries and is a growing health concern. In the most extreme form of NAFLD, the aberrant accumulation of fat can lead to inflammation, liver cancer, or organ damage so severe that

a liver transplant is required. **Cohen *et al.*** (p. 1519) review the current understanding of how NAFLD arises in humans, focusing on insights that have emerged from recent genetic and metabolic studies.

A Matter of Understanding

Quantum chromodynamics theory describes the internal structure of atoms—how quarks are held together to form protons and neutrons. Understanding matter relies on the ability to form a picture of the energy and thermodynamics involved in the interactions. This, in turn, requires the temperature scale to be set to allow the relative energies involved in the processes to be determined accurately. Using data from high-energy ion collisions, **Gupta *et al.*** (p. 1525; see the Perspective by **Müller**) formulate a computational method that allows the phase transition between solid matter and the point where it falls apart—the onset of the quark-gluon plasma—to be determined. The results should provide a clearer understanding of the fundamentals of how matter is put together.

We Are Stardust

In order to obtain the isotopic and elemental composition of solar matter, the NASA Genesis spacecraft captured samples of the solar wind outside of Earth's magnetosphere and returned them to Earth for laboratory analysis (see the Perspective by **Clayton**). **McKeegan *et al.*** (p. 1528; see the cover) and **Marty *et al.*** (p. 1533) describe the measurements of the oxygen and nitrogen isotopic composition of the Sun, based on laboratory analysis of the samples. Compared to the Sun, essentially all solar system materials are systematically enriched in the most heavy oxygen isotopes, ^{17}O and ^{18}O , and in the heavy nitrogen isotope, ^{15}N . Thus, Earth, the Moon, Mars, and the asteroids have oxygen and nitrogen isotopic compositions that are different from the average solar nebula from which they formed.

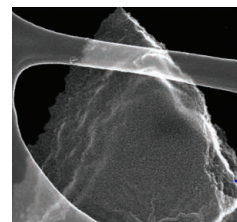
Fleecing Fluorocarbons

Synthetic carbon-fluorine bonds pose a conundrum. On one hand, they are turning up in a widening array of effective pharmaceuticals, as well as in a plethora of consumer products that require nonstick interfaces. On the other hand, they are consequently becoming a rising fraction of the waste stream, where they are very hard to break down. **Choi *et al.*** (p. 1545) discovered an unusual, indirect mode of activating these bonds, which may ultimately contribute to their

more facile manipulation into and out of various compounds. Specifically, hydrofluorocarbons such as CH_3F and $\text{C}_2\text{H}_5\text{F}$ react with an iridium complex through initial scission of a C-H bond, followed by rearrangements that result in metal-mediated cleavage of the C-F bond.

Tiny Capacitors, Big Possibilities

Capacitors work by storing electrical charge in conducting materials separated by a non-conductor. Capacitors charge and discharge more rapidly than batteries but typically hold much less charge, although in some applications supercapacitors can be used instead of rechargeable batteries. **Zhu *et al.*** (p. 1537, published online 12 May) fabricated a porous carbon material using microwaves to exfoliate graphite oxide that was then processed with potassium hydroxide to activate the carbon. Once treated, a network of 1- to 5-nanometer-width pores surrounded by single-atom layers of carbon was obtained, which had a low hydrogen and oxygen content and high electrical conductivity. Using acetonitrile as the electrolyte, a high gravimetric capacitance was obtained in a fabrication process that could be readily scaled up.



Bacteria Need Not Apply

The isotopic variation of some elements (deeply within rocks and sediments) can reflect the conditions at the time the layer was undergoing deposition. Large fractionations of iron isotopes, in particular, have recently emerged as a proxy for understanding which bacterial metabolisms were present and active during ancient Earth history. **Guilbaud *et al.*** (p. 1548), however, experimentally demonstrate that similar isotope fractionation values can be obtained abiotically through the formation of the iron-sulfide mineral pyrite. Because these fractionation values are similar to those measured in some of Earth's oldest rocks, iron isotopes may not be a reliable proxy for early bacterial iron-based metabolisms.

The Fountain of Youth?

Aging results in a slow deterioration of biological structures. Even budding yeast undergo replicative aging, with cells dying after producing a limited number of offspring. However,

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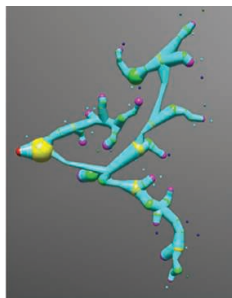
the effects of aging are not transmitted to offspring: The progeny of old and young organisms have similar life spans. **Ünal *et al.*** (p. 1554) find that, in budding yeast, life span is reset during sporulation. The life spans of spores derived from young and aged cells are essentially the same. Indeed, initiation of the sporulation program seems to be the trigger required to reset replicative life span. The transcription factor Ndt80 is associated with the rejuvenation effect, and its ectopic expression extended the life span of vegetatively growing cells.

Sorting Out the Centrosome

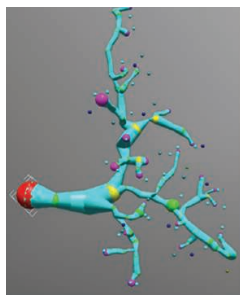
The centrosome is the paired organelle that organizes microtubules to form the mitotic spindle. The yeast centrosome (called the spindle pole body) is composed of 18 proteins. To help better understand how the centrosome is regulated, **Keck *et al.*** (p. 1557) made a comprehensive analysis of phosphorylation of the yeast centrosome proteins by mass spectroscopy. Almost 300 sites of phosphorylation were identified, about 100 of which occurred only during mitosis. The results may help point the way for further functional characterization of the more complicated human centrosome, which, with some 100 proteins, is likely to be regulated by a very large set of phosphorylation events.

Sleep on It

Nearly all animals above a certain level of complexity need to sleep. However, we still don't know why we have to sleep at all. By making use of the many genetic tools available in the fruit fly



and combining them with sophisticated behavioral and morphological analyses, **Bushey *et al.*** (p. 1576) and **Donlea *et al.*** (p. 1571) found that the dorsal fan-shaped body, a part of the central complex in the brain of *Drosophila*, could selectively induce sleep. Induction of sleep was important for long-term memory consolidation, and sleep itself was necessary for synaptic renormalization. Environmental enrichment resulted in a greater need for sleep, enhanced synaptic growth during wakefulness, and increased synaptic renormalization during



sleep. Furthermore, the increased need for sleep directly depended on synaptic growth during the awake period.

Light Control

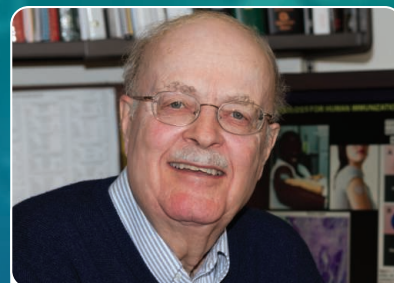
Melanopsin is a photopigment that triggers an intracellular calcium increase in response to blue light. **Ye *et al.*** (p. 1565; see the Perspective by **Chow and Boyden**) took advantage of calcium regulation of the transcription factor NFAT (nuclear factor of activated T cells) to design a synthetic signaling cascade that achieved light-inducible expression of transgenes under control of the NFAT promoter. Cells designed to display light-induced expression of glucagon-like peptide 1, a hormone involved in glucose homeostasis, were implanted into mice. Blue-light illumination of the implants attenuated glycemic excursions in type II diabetic mice. This approach of using light induction to provide tight regulation has potential applications in therapeutics and protein-expression technology.

Watch Out, Little Monkey

There has been considerable debate of whether the frontal eye field is associated with selective attention or with programmed eye movements. Performing electrophysiological recordings in monkeys, **Schafer and Moore** (p. 1568, published online 26 May; see the Perspective by **Roelfsema**) discovered that selective attention is a natural, untrained consequence of voluntarily driven changes in prefrontal neuronal activity. The activity of neurons in the frontal eye field can be increased and decreased voluntarily by using an operant conditioning, neurofeedback-type paradigm, similar to what has been classically observed in motor cortex. These voluntary changes in neuronal activity automatically resulted in spatially specific changes in visual perception and produced neural correlates of selective attention.

CREDIT: BUSHEY ET AL.

“A dream told me to do it.”



Carl R. Alving, M.D.
Chief of the Department of Adjuvant & Antigen Research, Division of Retrovirology at the Walter Reed Army Institute of Research
AAAS member

*Dr. Carl Alving
on his inspiration
for inventing
the vaccine patch.*

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Deborah Stipek is dean of the Stanford University School of Education, Stanford, CA 94305. E-mail: stipek@stanford.edu.

Education Is Not a Race

IN THE UNITED STATES AND ELSEWHERE, THE COMPETITIVE PRESSURES PLACED ON YOUNG PEOPLE in school are damaging many otherwise promising lives. In addition to generating debilitating anxiety and encouraging a culture of cheating, this competition takes the joy out of learning. The film *Race to Nowhere*, which continues to receive attention since its release a year ago, documents the unhealthy consequences of the competitive “teach to the test” climate that many U.S. students experience. The film, in which I was interviewed, puts in clear relief the pressures that youth are under to amass large numbers of Advanced Placement (college-equivalent) classes, win science fairs, excel in the arts and sports, and in other ways distinguish themselves from the competition for admission into a few select universities that parents and schools believe are critical for future success. Research on motivation makes it clear that focusing attention entirely on performance, whether grades or test scores, destroys whatever intrinsic interest the subject matter might have had.* There are certainly students whose passions spur them to realize their full potential in rigorous academic courses and other impressive activities. But how many potential Nobel Prize winners have written off science before the end of high school because their only exposure to the subject had been in test preparation courses rather than in classes that delved into meaningful questions? It doesn’t have to be this way, but change will require coordinated efforts at many levels.

Success in life does not require a degree from one of 10 universities. We need to evaluate U.S. high schools (pre-college education) on how well they help students find a college that matches their interests and goals, not on the proportion of students that they send to elite institutions. And the coveted universities need to demonstrate that they are interested in students who have a genuine passion for extending their educational experience, not merely in tallying items on resumé.

Many U.S. teachers also must change their approach to teaching. Extensive research shows that students will become more emotionally engaged (and even passionate) if simple principles are followed: if the subject matter is connected to students’ personal lives and interests; if students have opportunities to be actively involved in solving or designing solutions to novel and multidimensional problems, doing experiments, debating the implications of findings, or working collaboratively; if students have multiple opportunities to earn a good grade (by rewriting papers or retaking tests); if attention is drawn to the knowledge and skills that students are developing, not to grades or scores; and if all learning and skill development is celebrated, whatever the level.

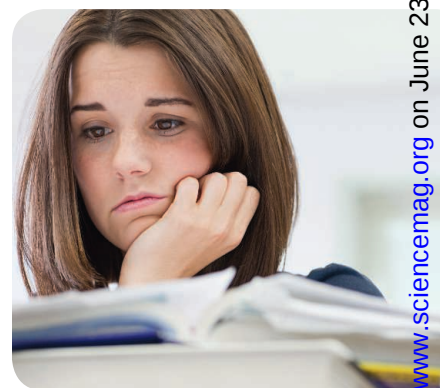
Schools must create homework policies to ensure that diligent students aren’t kept up late into the night; schedule some spacing between major tests and offer ample opportunities for students to get extra help; make sure that at least one adult is paying attention to every student’s emotional needs; provide parent education on the advantages of a broad array of potential colleges; survey students regularly on the sources of their stress and make sure that this feedback informs policies; and offer opportunities for students to pursue academic interests unencumbered by performance concerns, such as in independent studies or clubs.

The world is rapidly changing. Problem-solving skills and critical analysis have become infinitely more important than being able to answer the typical questions given on standardized tests. A valuable science of teaching and learning exists that should guide efforts to improve students’ interest, engagement, and intellectual skills, as well as reduce the debilitating stress that is becoming epidemic.** Only by paying attention to what we know can we make the changes that youth need to lead healthy and productive lives.

— Deborah Stipek

10.1126/science.1209339

**Motivation in Education: Theory, Research, and Applications* (Pearson/Merrill Prentice Hall, ed. 3, Upper Saddle River, NJ, 2007). **National Research Council, *Engaging Schools: Fostering High School Students’ Motivation to Learn* (National Academies Press, Washington, DC, 2003).



PSYCHOLOGY

Channeling Euclid

Behavioral research has explored how human and animal understanding of space reflects principles of Euclidean geometry. Though perception of the environment can affect many mental processes, Kant argued for an *a priori*, innate spatial intuition. Such intuition might underpin the ability to understand geometric concepts that are not observable in nature, such as an infinitely thin line or infinitely large plane. To address the universality of Euclidean concepts, Izard *et al.* tested a population indigenous to the Amazon, the Mundurucu, who have no formal training in geometry. This group of both children and adults was compared to mathematically educated U.S. adults, comparably aged French children, and younger U.S. children. Among other queries, people were shown dots and lines, representing villages and paths, projected on a plane or sphere, and asked whether a line could be drawn through a point and never cross another line. The Mundurucu responses were similar to those of U.S. adults and French children, reflecting strong application of Euclidean principles. The younger U.S. children responded similarly but were more error-prone. Across cultures, people were biased to erroneously apply Euclidean principles to spheres. The authors propose that new research explore whether understanding of abstract geometry is innate but only emergent at a certain time in development, or whether it is learned as a result of experiences so general that all humans are exposed to them. — BW

Proc. Natl. Acad. Sci. U.S.A. **108**, 9782 (2011).



MATERIALS SCIENCE

Nanoparticles Make the Cut

It has long been known from *ex situ* studies that metal nanoparticles can catalyze reaction of oxygen with graphite surfaces and create grooves or channels. Such reactions could be used for patterning graphene sheets. Booth *et al.* have studied the dynamics of silver nanoparticles on suspended monolayer and bilayer graphene sheets in a transmission electron microscope. They imaged these samples at temperatures from 600 to 850 K and partial pressures of oxygen over the sample from about 30 to 100 millitorr. The nanoparticles cut channels along $\langle 100 \rangle$ crystallographic directions, but some fluctuations of motion normal to the channel direction were also observed. The nanoparticles did not move at a constant speed. Instead, their velocity profile was erratic, and the start-stop motion was better described by a Poisson distribution. — PDS

Nano Lett. **11**, 10.1021/nl200928k (2011).

BIOCHEMISTRY

Breakdown Breakthrough

Lignin is an organic polymer that binds tightly to cellulose fibers in plant cell walls, imparting rigidity and strength. The conversion of plant biomass into biofuels has been a challenge, in

part because lignin hinders the degradation of cellulose into sugars that can be fermented. One approach is to use enzymes that break down lignin. Although such enzymes have been characterized in fungi, these microorganisms have been difficult to exploit commercially. Some bacteria secrete lignin-degrading enzymes, but the details are not well understood. The identification of a bacterial lignin-degrading gene would allow large-scale production of the enzyme in an organism that is easy to genetically manipulate and grow. Ahmad *et al.* used a bioinformatics approach to identify a gene in the soil bacterium *Rhodococcus jostii* that encodes a lignin-degrading peroxidase (DypB). In the presence of manganese salts, recombinant DypB expressed and purified from *Escherichia coli* showed degradation activity toward lignin, lignocelluloses from wheat straw, and synthetic model compounds. It is not clear how DypB is secreted from *R. jostii*, but the *dypB* gene is adjacent to a gene that encodes encapsulin, a shell-forming protein. The authors speculate that the genes may be coupled, and that an encapsulin nanocompartment could facilitate secretion. — LC

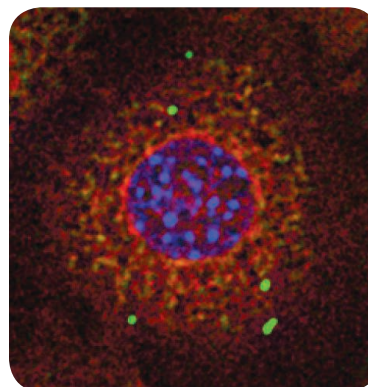
Biochemistry **50**, 5096 (2011).

IMMUNOLOGY

Viruses Are (I)FIT To Be Tied

The immune system is constantly surveying the body for signs of infection, but how can it distinguish viruses from self? Viruses can be distinguished from self because their nucleic acids contain specific characteristics, such as the triphosphorylated RNA (PPP-RNA), that are not found in the nucleic acids of host cells. The molecules that recognize these viral structures, however, are still

being identified. Pichlmair *et al.* carried out a screen to identify proteins that interact with PPP-RNA and identified several members of the IFIT family of interferon-stimulated proteins. In response to antiviral interferons, IFIT proteins formed a molecular complex with other family members and



RNA-binding proteins. Subsequent biochemical and genetic analysis focused on IFIT1 and found that, although it did not appear to be involved

Continued on page 1485

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in the initial detection of the virus, it was highly induced in response to antiviral interferons and was required for keeping viral growth in check in cultured cells and in mice infected with vesicular stomatitis virus. Although IFITs have been previously associated with inhibition of protein translation, the authors presented data consistent with IFIT1 functioning by sequestering viral nucleic acids within the cell. — KLM

Nat. Immunol. **12**, 10.1038/ni.2048 (2011).

CHEMISTRY

Phosphorus Ins and Outs

Both amines and phosphines are shaped like pyramids. The difference is that amines rapidly undergo so-called umbrella inversions, whereby the three groups pushed downward by the electron lone pair at the nitrogen vertex suddenly point upward (or vice versa). In phosphines, the inversion is much slower: At room temperature, the three groups bound to P tend to stay put. Stollenz *et al.* were therefore initially puzzled by the behavior of a diphosphine in which three flexible $(CH_2)_{14}$ chains bridge two P atoms: Facile conversion was taking place between the isomer with both electron pairs facing in and the one with both pairs facing out. Some study of models convinced them of a rather distinct mechanism, in which one of the methylene chains was passing through the ring bounded by the other two, in effect turning the compound inside out. To bolster this hypothesis, they heated the compound to high enough temperature to induce the more common umbrella inversions and isolate in/out isomers, and then verified that these new isomers could not convert to the in/in and out/out variants at room temperature—a result in accord with the topological restrictions of the inside-out pathway. — JSY

Angew. Chem. Int. Ed. **50**, 10.1002/anie.201100893 (2011).

DEVELOPMENT

It's Complicated

Puberty in humans responds to factors as varied as nutritional status and social interactions. But sooner or later, pulses of gonadotropin-releasing

hormone begin and puberty ensues. Previous studies had implicated the neuropeptide kisspeptin in initiating puberty, but Mayer and Boehm now show that puberty initiation may be more complicated. Studying mice, the authors selectively ablated neurons that expressed kisspeptin, or its receptor, in young or mature mice. Although deletion of the genes encoding kisspeptin or its receptor result in infertility and deficient gonadal development, toxin-mediated ablation of neurons that express kisspeptin did not. If the ablation occurred in young mice, the mice went through puberty and reached fertility, although with smaller-than-normal gonads. If the ablation occurred in mature mice, these mice became infertile. The differential sensitivity of mice to kisspeptin gene deletion compared to deletion of kisspeptin-expressing neurons indicates that there are probably redundancies in the puberty-initiating machinery, which, given the importance of this process to the success of the species, may make sense. — PJH

Nat. Neurosci. **14**, 704 (2011).

IMMUNOLOGY

Estrogen Receptor's Two Faces

Many autoimmune diseases, including multiple sclerosis (MS), are more prevalent in women.

This, coupled with prior findings implicating a role for the estrogen receptor (ER) in MS, prompted Saijo *et al.* to uncover the underlying molecular mechanisms. After determining that microglia, resident myeloid cells in the brain, primarily express ER β , the authors showed that depending on the ligand, signaling through ER β could either induce or inhibit proinflammatory gene expression. 17 β -estradiol, which is more prevalent in women, drove expression of proinflammatory genes, whereas 5-androsten-3 β ,17 β -diol (ADIOL) inhibited them. This occurred because ADIOL, but not 17 β -estradiol, led to the recruitment of CtBP corepressor complexes, which functioned with ER β and the transcription factor AP-1 to shut down proinflammatory gene expression. In women, this pathway may be antagonized because of increased amounts of 17 β -estradiol, which competes with ADIOL for binding to ER β and does not induce the recruitment of CtBP. Synthetic ligands that signaled similarly to ADIOL were protective and therapeutic in a mouse model of MS, which suggests that this pathway may be a useful target for therapeutic intervention. — KLM

Cell **13**, 584 (2011).

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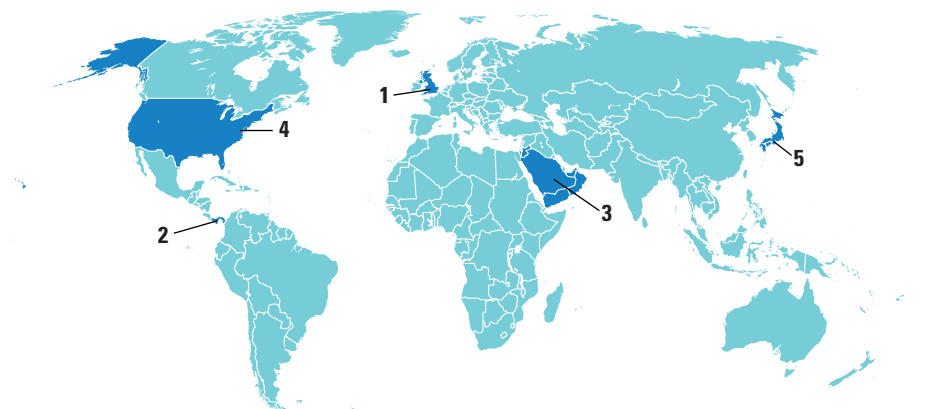
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U.K. Abandons DNA, Isotope Testing of Asylum Seekers

The U.K. Border Agency (UKBA) has ended its widely scorned investigation into DNA and isotope testing of human tissues as a means to verify the nationality claims of asylum seekers (*Science*, 2 October 2009, p. 30). *The Times* broke the news 17 June, labeling the so-called Human Provenance Pilot Project an “expensive flop.” UKBA reportedly spent £190,000 on the effort.

After geneticists pointed out in 2009 that DNA testing might reveal ancestry but could not prove nationality, UKBA suspended the pilot project, but later resumed taking tissue samples from asylum seekers on a voluntary basis. UKBA also said that any data collected during the pilot project would not be used in asylum decisions.

The agency now says the pilot project ended in March, and it doesn’t anticipate publishing the collected data or any evaluation of the effort. http://scim.ag/_nationality

Panama 2

Bad News for Central American Frogs

A fungal disease blamed for the disappearance of amphibians around the world is continuing its relentless march through Panama and has reached the border of one of Central America’s largest remaining wilderness areas, according to the Smithsonian Institution.

Doug Woodhams from the University of Zurich in Switzerland has found two infected frogs near the Darién province, where the

local national park is a World Heritage site. In 2007, he found none. About 100 of Panama’s 200 species of frogs call Darién home and half could disappear once the disease hits, says Brian Gratwicke, a biologist at the Smithsonian Conservation Biology Institute in Front Royal, Virginia. Gratwicke says the park represents one of the last reservoirs to collect uninfected Panamanian frogs for the captive breeding program he runs.

But Panama is not lost yet, cautions Karen Lips, an ecologist at the University of Maryland, College Park, who has been tracking the disease’s spread. Another recent survey found no signs of infection at the border between Columbia and Darién. “We know [the fungus] is on the move, but there are still healthy populations,” she points out.

Arabian Peninsula, Israel, and Jordan 3

Arabian Oryx Back From the Brink

Almost 40 years ago, the last wild Arabian oryx (*Oryx leucoryx*), a large, cream-colored antelope with striking black horns, was shot by a hunter in the deserts of Oman. But last week, conservationists announced that the oryx, which may have led to the legend of the unicorn, has been successfully restored to its native habitat on the Arabian Peninsula. It’s the first time that scientists have achieved such a remarkable turnaround for a species once declared extinct in the wild.

Today, about 1000 wild oryx, descendants of animals bred in captivity, roam the deserts of Saudi Arabia, Israel, the United Arab Emirates, Oman, and Jordan. The populations are not connected, however, nor are there corridors yet to link the scattered herds, some of which number less than 40 animals.

Unregulated hunting and poaching led to the animals’ original decline. “To the extent [that illegal hunting] is controlled, the



population should grow fairly rapidly,” says Michael Hutchins, executive director of The Wildlife Society in Bethesda, Maryland. http://scim.ag/_oryx

Washington, D.C. 4

Supreme Court Rejects ‘Nuisance’ Argument Against Plant Emissions

The U.S. Supreme Court has rejected the argument that utility companies can be sued to force them to reduce their greenhouse gas emissions because those emissions constitute a threat to society. In an 8-0 ruling, the high court said 20 June that the U.S. Environmental Protection Agency already has that authority under existing federal laws and that “there is no room for a parallel track” for a lawsuit alleging a common law violation.

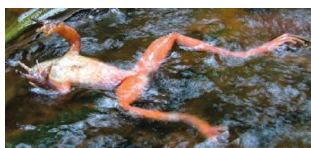
The case, *American Electric Power v. Connecticut*, was brought in 2004 by several states and conservation groups against five large utility companies whose fossil-fuel power plants generate 25% of U.S. emissions from such sources. A district court had dismissed the suit but an appellate court ruled that the plaintiffs had standing because of an old doctrine that pollutants constitute a public nuisance. http://scim.ag/_nuisance

Kobe, Japan 5

‘K Computer’ Is Named Most Powerful

Move over, China. Japan’s “K Computer” is now the fastest supercomputer in the world. On 20 June, K was ranked number one in the TOP500 list of the world’s supercomputers, performing three times as fast as its Chinese rival and the previous champion, Tianhe-1A.

The TOP500 list is updated twice a year and ranks how quickly computers solve a standard mathematical equation. K, built by Fujitsu and located at the RIKEN Advanced Institute for Computational Science in Kobe, can perform 8.2 quadrillion calculations per second, equivalent to linking about 1 million desktop computers. That performance is still shy of the target *kei*, or 10 quadrillion, calculations for which the supercomputer was named. This is the first time Japan has topped the list since 2004.



NEWSMAKERS

Zoghbi Wins Gruber Prize



This year's Gruber Prize in Neuroscience goes to pediatric neurologist and geneticist Huda Zoghbi of Baylor College of Medicine in Houston, Texas. The \$500,000 award honors Zoghbi's pioneering work

on the genetic and molecular mechanisms of several neurological disorders.

In 1993, Zoghbi co-discovered a genetic defect responsible for spinocerebellar ataxia type 1, a neurodegenerative disorder that robs affected individuals of movement. Her laboratory reported another major breakthrough in 1999 when it discovered that mutations in the *MECP2* gene cause Rett syndrome, an autism spectrum disorder that affects girls. More recently, Zoghbi's group has built on these findings with several important contributions to understanding the molecular mechanisms of these and other disorders.

"What stands out about Dr. Zoghbi's discoveries is that the original inspiration for her science was her clinical observations—and her determination to 'go to the bench' to solve the mystery of the disorder," said Carol Barnes, the neuroscientist who chaired the Gruber neuroscience selection committee.

Ingenuous Inventor

Materials scientist, applied physicist, and entrepreneur John Rogers, 43, of the University of Illinois, Urbana-Champaign, has won the \$500,000 Lemelson-MIT Prize for 2011. The award is given to a scientist whose inventions offer innovative solutions to real-world problems.



Rogers's inventions tend to be hybrids that blend biology, electronics, nanomaterials, and optics. They include a photovoltaic technology that uses tiny ball lenses to focus sunlight onto solar cells. Rogers and colleagues are developing this technology, which produces a highly efficient conversion of sunlight to electrical energy, through Semiprius, a company they launched in 2006.

Rogers launched another company, mc10, in 2008 to commercialize his designs for "stretchable electronics," silicon-and-rubber medical devices that can curve and twist to map the electrical properties of the heart or the folds of the human brain.

Rogers, who also directs the National Science Foundation's Nanoscale Science and Engineering Center, holds 80 patents or patent applications. "This prize is kind of a recognition of the collective set," he says.

"John Rogers takes the cross section of scientific and technological development for practical application to a new level," said Michael J. Cima, the faculty director of the Lemelson-MIT Program. "The work is striking in its novelty and marketability."

Middle Schoolers Awarded Prize for Patent

Six Iowa Girl Scouts have been awarded \$20,000 from the X PRIZE Foundation to help obtain a patent on their original design for a prosthetic hand device. The middle schoolers, ages 11 to 13, invented the device as part of an international biomedical engineering challenge sponsored by the FIRST LEGO League (FLL).

This year's contest was the first competition with a monetary prize incentive in FLL's 11-year history. There were 179 submissions from 56 countries. At the award ceremony 16 June in Washington, D.C., FIRST Founder Dean Kamen said "the really big idea here is spreading the idea that young kids ... can be, even at their age, a big part



of solving the world's problems."

The winners were inspired by 3-year-old Danielle Fairchild from Georgia, who was born without fingers on her dominant hand. After connecting with Danielle's mother through an Internet support group, the team designed a prototype of the device, dubbed BOB-1. Their winning design, according to Kamen, was selected based on its simplicity and resourcefulness, and has already allowed the toddler to write for the first time.

FINDINGS

The Iceman's Last Meal

Ever since he was discovered in the Italian Alps in 1991, Ötzi the 5200-year-old Iceman has been dishing out information about Neolithic life. Scientists at the 7th World Congress on Mummy Studies 12 to 16 June reported new findings on the Iceman's dietary and dental habits, and even his eye color. >>



Mercury Reveals Some Surprises

The solar system's innermost planet may look like a dead ringer for Earth's moon, but the scientists getting the closest look ever at Mercury want you to know one thing: Mercury is not like the moon or any of the other rocky planets—Earth, Mars, or Venus. At a NASA press conference 16 June, researchers outlined several distinctive mercurian aspects gleaned from the MESSENGER spacecraft's first 3 months in orbit around Mercury. Perhaps the most fundamental is the abundance of elements on Mercury's surface that can be easily boiled out of hot rock, including sulfur and potassium. That volatile-rich rock rules out the possibility that an Earth-size Mercury had its outer parts blasted away by a nascent sun. With all systems go, MESSENGER has 9 months left in its planned mission. http://scim.ag/_mercury

>>FINDINGS



Researchers led by microbiologist Frank Maixner of the Institute for Mummies and the Iceman in Bolzano, Italy, reported at the meeting that they had sequenced the DNA of animal fibers in the Iceman's stomach to reveal his last meal: the meat of an Alpine ibex. New three-dimensional images of the Iceman's teeth by dentist Roger Seiler and anatomist Frank Rühli of the Center for Evolutionary Medicine at the University of Zürich, meanwhile, show that the Iceman was plagued by both periodontal disease and cavities.

And a team led by geneticist Angela Graefen of the Institute for Mummies and the Iceman reported sequencing the Iceman's whole genome, despite the highly fragmented nuclear DNA. The genome

has already revealed some surprises. One preliminary finding shows that the Iceman probably had brown eyes rather than the blue eyes found in many facial reconstructions done by artists. http://scim.ag/_iceman

Dinosaurs May Have Been Warm and Cuddly

Dinosaurs' reputation as cold-hearted beasts may be undeserved. By analyzing teeth from fossils of the huge four-legged dinosaurs *Brachiosaurus* and *Camarasaurus*, researchers have found hints that strengthen the idea that some dinosaurs may have been warm-blooded, or at least better at conserving body heat than modern reptiles are.

The researchers measured isotopes of chemical elements in tooth enamel from 11 fossils dug up in Oklahoma, Wyoming, and Tanzania. The lower the temperature at which the enamel forms, the more the isotopes carbon-13 and oxygen-18 tend to bond or clump together. The bond structure in the dino teeth suggested the enamel formed between 36° and 38°C, similar to mammalian body temperature. It's not the final word on warm-blooded dinosaurs, the authors say in a paper published online this week in *Science*. If these "gigantotherms" were this warm, they

BY THE NUMBERS

\$547.9 million Amount NASA plans to pay to help cover the costs of closing out the space shuttle program and paying off pensions of United Space Alliance's 11,000 workers and retirees.

704 Number of brain injuries in *Asterix* comics, according to a study in *Acta Neurochirurgica*.

25 Number of academic institutions, including the top five neuroscience programs in the United States, that have formed research collaborations with brain teaser Web site Lumosity.

would have had to release heat through some unknown mechanism to avoid overheating, possibly through their long necks and tails.

Neutrino Discoveries to Come?

Sometimes it's not the results themselves but the prospects they raise that jazz scientists. Physicists in Japan have directly observed particles called muon neutrinos transforming into others called electron neutrinos. Obtained by shooting trillions of muon neutrinos from an accelerator lab in Tokai to the subterranean Super-Kamiokande particle detector 295 kilometers away, the observation from the T2K experiment was expected. Nevertheless, it excites physicists because the effect appears to be so large that future experiments may have a relatively easy time spotting other phenomena. "If this result holds up, ... we've got kind of a fun decade ahead of us in neutrino physics," says Mark Messier, a neutrino physicist at Indiana University, Bloomington.

In particular, physicists are keen to compare the rate at which a muon neutrino becomes an electron neutrino to that at which a muon antineutrino becomes an electron antineutrino. Any difference would violate a symmetry between matter and antimatter known as charge-parity symmetry, and might help explain why the universe evolved to contain so much of the former and so little of the latter. However, Messier says, such an experiment is at least a decade away. http://scim.ag/_neutrino

Random Sample

Aaand They're Off!

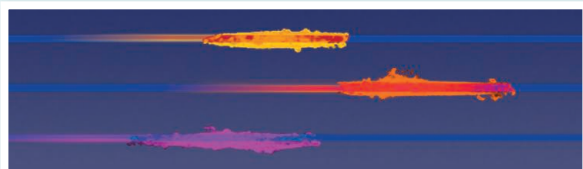
Cell biologists and racing enthusiasts, heads up! There is still a week left to enter the First World Cell Race, which pits hundreds of types of cells against each other to find out which are the fastest.

Cell biologists Matthieu Piel and Ana-Maria Lennon-Duménil of Institut Curie in Paris and physicist Manuel Théry of the French Atomic Energy Research Center in Grenoble, France, dreamed up the race at last year's annual meeting of the American Society for Cell Biology. "We thought it would be nice to see which was the fastest of all cells," Piel says.

The trio has designed "racetracks" coated with a substrate to which the cells attach. Through the end of July, participants may submit cell cultures to one of six laboratories around the world, which will film the cells' progress throughout July and August. The cells that migrate the fastest along 100 micrometers of track will be declared the winner.

In addition to fun, the race offers a chance to compare the cells' migration strategies under identical conditions and create a sort of global library of cell migration strategies, Théry says. All of the race data will be posted on a public Web site for cell migration researchers.

Two types of speedy cells—metastatic cells (cancer cells that have spread) and white blood cells—have especially good odds, creating a natural "good versus evil" rivalry, Piel says. But the wide-open field, which includes mutant cells and cells with knocked out genes, could well pave the way for a dark horse. Even if your cells aren't the fastest, it's important to enter the race, Piel says: "People only seem to want to participate if they think their cells have a chance to win." For more information, check out www.worldcellrace.com.



BIOSECURITY

Panel Selects Most Dangerous Select Agents

After the Federal Bureau of Investigation (FBI) last year declared that the 2001 anthrax letter attacks had been perpetrated by a U.S. Army researcher, government officials and biomedical scientists stepped up the debate over what can be done to better guard against the nefarious use of dangerous pathogens and toxins, collectively known as select agents. Last week, a federal panel called for tightening security measures on a select group of select agents and loosening research restrictions on others. The proposal has by and large been welcomed by the biomedical community, but some are balking at its recommendations for enhanced screening and monitoring of researchers who work with the most dangerous pathogens.

The Federal Experts Security Advisory Panel, led by biodefense expert George Korch of the U.S. Department of Health and Human Services (HHS) and veterinarian Gregory Parham of the U.S. Department of Agriculture, was formed after President Barack Obama issued an executive order in July 2010 announcing that the government would overhaul the select agent program. The panel's report lists 11 bacteria and viruses that should be designated as Tier 1 agents: a rogue's gallery of microorganisms that would be subjected to higher security standards than other pathogens and toxins used in biomedical research. At the same time, the panel recommends dropping 19 pathogens and six toxins from the broader list of 82 agents that are currently governed by the select agent program.

"We were provided with briefings from outside groups with opinions ranging from 'Don't regulate anything' to 'Don't remove anything from the list,'" Korch says. "We had to strike a balance."

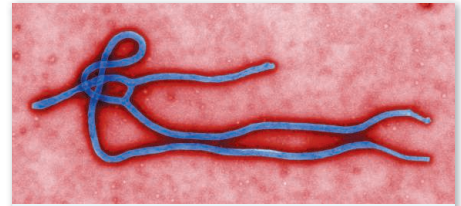
The Tier 1 agents were selected on the basis of several criteria, such as prior use of the pathogen as a biological weapon, the possibility that it might cause mass casualties, and the potential economic impact in the event of an outbreak. Some pathogens, such as those for Ebola and anthrax, were obvious Tier 1 agents, but a few debates did arise, among them whether to include botulinum toxin-producing strains. "These strains

are available environmentally, and Bt toxin as a commercial product is fairly ubiquitous," Korch says, explaining why the panel had initially left the item off the list. What changed that decision, he says, was intelligence information suggesting that U.S. enemies had looked into using the pathogen as a weapon. Using the same criteria, the panel determined that agents like the European subtypes of tick-borne encephalitis (see list) did not represent significant threats and could be taken off the select agent list.

Ron Atlas, a microbiologist at the University of Louisville in Kentucky and co-chair of the American Society for Microbiology's biodefense committee, welcomes the Tier 1 approach. "By focusing on the most dangerous agents, there is a greater likelihood of ensuring the security of those agents within U.S. laboratories," he says. And removing some of the less hazardous agents, Atlas adds, "will reduce the regulatory burden on U.S. laboratories without increasing the risk of bioterrorism."

More controversial are the panel's recommendations that are aimed at reducing the so-called insider threat: the possibility that a rogue researcher might use select agents to cause deliberate harm, as the FBI concluded U.S. Army researcher Bruce Ivins did with anthrax. The panel recommends giving those screening a researcher's background access to databases of individuals judged by a court or other legal authority to be mentally defective or unstable; Ivins had a documented history of mental problems, but they were largely ignored.

The panel recommends that the Department of Justice study the feasibility of asking foreign nationals who want to do research involving Tier 1 agents to furnish a criminal background check conducted by authorities in their home country, and having those working with such agents be screened against terrorist databases on an ongoing basis rather than the current norm of once every 5 years. The panel also proposes periodic checks of credit statements and other financial records for any personnel with access to Tier 1 agents. And the panel says it is still exploring the use of behavioral



AGENTS DEEMED TIER 1

Bacillus anthracis
Burkholderia mallei
Burkholderia pseudomallei
 Ebola virus (pictured)
 Foot-and-mouth disease virus
Francisella tularensis
 Marburg virus
 Variola major virus
 Variola minor virus
Yersinia pestis
 Botulinum neurotoxin and botulinum toxin-producing species of *Clostridium botulinum*

AGENTS RECOMMENDED FOR REMOVAL FROM SELECT AGENT LIST

Cercopithecine herpesvirus 1 (Herpes B virus)
Coccidioides posadasii
Coccidioides immitis
 Eastern equine encephalitis virus, South American genotypes
 Flexal virus
 Tick-borne encephalitis viruses, European subtypes
 Venezuelan equine encephalitis virus, enzootic subtypes ID and IE
 Akabane virus
 Bluetongue virus
 Bovine spongiform encephalopathy
 Camel pox virus
Ehrlichia ruminantium
 Goat pox virus
 Japanese encephalitis virus
 Malignant catarrhal fever virus
 Menangle virus
Mycoplasma capricolum, subsp. *capripneumoniae*
 Sheep pox virus
 Vesicular stomatitis virus

TOXINS RECOMMENDED FOR DELISTING

Clostridium perfringens epsilon toxin
 Conotoxin
 Diacetoxyscirpenol
 Shiga toxin
 Shiga-like ribosome inactivating proteins
 T-2 toxin

assessment tools to determine if researchers are psychologically fit to work with pathogens such as anthrax.

Some university and lab administrators argue that such increased scrutiny will drive talent away without making labs more secure. Nancy Connell, who directs a bio-defense lab at the University of Medicine and Dentistry of New Jersey in Newark, says supervisors and local officials, not the administrators of the federal select agent program, are in the best position to ensure the reliability of personnel working in their

labs. “I do this at a very personal level by keeping close track of who is in the lab when and how they are doing in their personal lives,” she says. “I give them warning when I hire them that I will be keeping track of their emotional state, for safety as well as security reasons. If someone is under significant emotional stress and distracted in any way, I redirect their lab responsibilities for a while until things settle down.”

Richard Ebright, a microbiologist at Rut-

gers University in Piscataway, New Jersey, who has been a frequent critic of the current select agent program, says the panel should have recommended video monitoring of work areas—currently left up to lab managers—and a requirement that researchers never work alone in a select agent lab. Laura Kwinn, the panel’s executive secretary and a science policy adviser at HHS in Washington, D.C., says the group rejected those suggestions after careful analysis.

The panel’s work is not over: A working group within it, led by officials from the Department of Homeland Security, is developing recommendations to make select agent labs more secure physically. Once the government has mulled over all of the recommendations, it will issue a notification spelling out proposed changes to the select agent program. That is expected to happen in the coming months.

—YUDHIJIT BHATTACHARJEE

BIOMEDICINE

NIH’s Secondhand Shop for Tried-and-Tested Drugs

Although the U.S. National Institutes of Health (NIH) has made waves with a proposed new center aimed at translational research, so far the main innovation has been to put scattered existing programs under the same roof. But this month NIH Director Francis Collins unveiled something fresh: an effort to persuade drug companies to open up their troves of abandoned drugs to academics, who would look for new uses.

University in St. Louis, university researchers have access to a database of 500 Pfizer drugs and failed candidates that they test in animal models.

But NIH officials think there’s merit in a more systematic effort. One reason is efficiency, NIH Associate Director for Science Policy Amy Patterson explained to the NIH board this month. Although only 1 in 10,000 potential therapeutic compounds will

As for logistics, the agency has made a small start. In April, NIH’s intramural Chemical Genomics Center unveiled a public database listing all 8000 or so approved drugs along with structural data (*Science Translational Medicine*, 27 April, <http://scim.ag/chem-genome>). Researchers can apply to have the center test their cell or molecular assays against the drugs to look for “hits,” or possible biological activity.



NIH DRUG REPURPOSING		
Drug	Initial Indication	Subsequent Indication
AZT	Antineoplastic	HIV/AIDS
Ceftriaxone	Bacterial infection	Amyotrophic lateral sclerosis
Hydroxyurea	Cancers	Sickle cell anemia
Metformin	Type 2 diabetes	Breast cancer
Pioglitazone	Type 2 diabetes	Hepatic steatosis
Raloxifene	Osteoporosis	Breast cancer
Tamoxifen	Breast cancer	Bipolar disorder

Double duty. NIH researchers have found new uses for several therapeutics.

The drug rescue and repurposing project, Collins told his advisory committee earlier this month, is a concrete example of what the National Center for Advancing Translational Sciences (NCATS) will do. The project shows how NCATS could be “quite game-changing,” he added. It would be a “comprehensive effort to identify appropriate abandoned compounds,” “match partners,” and “make data and resources available,” Collins explained in a comment in the June issue of *Nature Reviews Drug Discovery*.

Finding new uses for drugs is not a novel idea, however. Thalidomide, a drug for morning sickness that was taken off the market because it caused severe birth defects, later found new life as a treatment for leprosy and multiple myeloma. And many companies already have drug-repurposing efforts. Academia is also getting involved. For example, under a 1-year-old deal between Pfizer and Washington

have yielded a wealth of new disease targets.

Most of the therapeutic mother lode lies in the thousands of abandoned compounds in pharmaceutical companies’ vaults. Companies face obstacles to sharing data on these drugs, however, Patterson noted. They may have to digitize paper records, and often the company’s expert on that drug has moved on. Testing can be a worry because it could reveal new safety problems for drugs on the market. In addition, intellectual property agreements need to be worked out.

NIH is working on a draft “master agreement” between companies and academics that addresses intellectual property and issues such as data sharing. NIH may also propose modifying patent laws to give companies financial incentives, Collins says. But this would be a “tremendous undertaking,” according to Patterson; for now, the agency is looking for leeway in existing laws.

For unapproved drugs, Patterson says, NIH envisions a system of databases that would allow researchers to “window-shop” by viewing public data. If they see a compound that interests them, they might access a company’s proprietary data through service companies.

NIH hopes to complete the model master agreement within 6 to 8 months, Patterson says. The drug rescue and repurposing project will be led by a team at NCATS as “an integral part of what NCATS does,” Patterson says. Some promising projects will then be handed off to other NIH institutes.

Some academic researchers are enthusiastic. “Provided all the details can be worked out, I think it’s a win for all sides,” says pharmacologist Bryan Roth of the University of North Carolina, Chapel Hill.

Several industry researchers who attended an April roundtable that discussed the plan did not respond to a request for comment. But William Chin, a former research leader at Eli Lilly who is now executive dean for research at Harvard Medical School in Boston, says companies should be interested because often their own repurposing efforts haven’t been very fruitful. NIH’s “hope is that the ‘wisdom of the crowd’ will result in a new way to use an old molecule,” he says. “If the majority of companies joined in this effort, it potentially would generate a stockpile of potential assets” and a few “valuable therapeutics.”

—JOCELYN KAISER

LUNAR SCIENCE

Apollo Physicist Launches Noisy Dustup Over Old Moon Data

Whipping around the moon in the solar system's loneliest spaceship, Apollo 8 astronaut James Lovell saw something in 1968 that he shouldn't have: a gentle illumination, like a sunrise or sunset on Earth, hovered where the sun's light cast its sharp shadow on the moon's surface. Yet the moon has no atmosphere to catch the sun's rays and create such a spectacle.

Other astronauts and photos from Surveyor moon landers confirmed the horizon glow. So scientists hypothesized that lunar dust was picking up enough of an electric charge from cosmic rays or the solar wind to drive it tens of kilometers into the otherwise vacant lunar sky and cause the light show. A particle monitoring instrument, the Lunar Ejecta and Meteorites (LEAM) experiment, placed on the moon 4 years later seemed to provide corroborating data.

But now a former Apollo physicist is threatening to take the glow off this explanation. Brian O'Brien, who helped design dust monitors for Apollo 11, 12, 14, and 15, argues in a review published online recently by *Planetary and Space Science* that much of the LEAM data were not detections of charged lunar dust particles but instead electrical interference generated by the Apollo Lunar Surface Experiments Package (ALSEP) instruments parked 7.5 meters away from LEAM.

That claim has roused other Apollo scientists and engineers out of retirement. "I'm amazed that *Planetary and Space Science* accepted Brian's paper," says Lynn Lewis, the ALSEP systems manager and chair of a group trying to find missing data from the package.

The nostalgic dustup comes as NASA prepares for the 2013 launch of the Lunar Atmosphere and Dust Environment Explorer (LADEE), a lunar satellite whose instruments could resolve whether such charged particles exist and how they move above the moon. Many of the participants will face off at next month's 4th NASA Lunar Science Forum at Ames Research Park in California.

Lunar dust, and dust on other rocky planets and asteroids, is important to space exploration for several reasons. Researchers who count craters on airless, waterless bodies or analyze the chemistry of their rocky surfaces to try to estimate their age and composition need to know how much

dust is flying around to calibrate measurements, says physicist Eberhard Grün of the Max Planck Institute for Nuclear Physics in Heidelberg, Germany, and the University of Colorado, Boulder. Dust creates practical problems, too. Surveyor 3, a robotic lander sent to the moon in 1967, showed signs of serious dust abrasion when it was collected 2 years later by Apollo 12 astronauts. Dust also darkened the moon explorers' suits, heating them beyond the cooling systems' capacity, and the particles' sharp, glassy edges caused leaks in the suits.

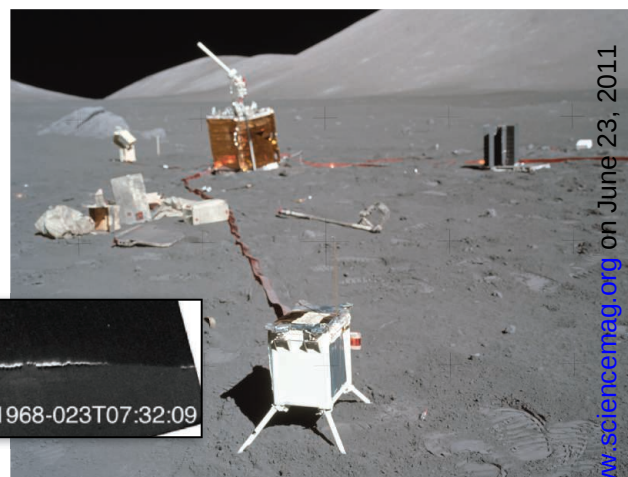
NASA designed the LEAM experiment, installed near the Apollo 17 landing site in December 1972, to detect a predicted continual rain of fast-moving micrometeorites and the fine lunar dust they kicked up upon impact. Yet LEAM recorded the most activity at lunar sunrise or sunset. The types of events recorded were odd as well: They saturated the instrument's sensors and lasted longer than they should have if caused by fast interplanetary particles.

Lunar scientists later concluded that LEAM was seeing slow-moving charged dust particles close to the ground. Perhaps as sunlight struck the moon and charged some dust particles but not neighboring ones still in the shade, the difference in electrical charges would drag lighter particles around the surface or even into the sky, they hypothesized. If such particles also floated kilometers higher, then the LEAM data might support the idea that the horizon glow was lunar dust.

But in his new paper, O'Brien, who was at Rice University in Houston, Texas, at the time of the Apollo missions, questions whether the LEAM data represent dust. For example, he says that too many of the unexpected signals arrive in well-ordered bursts to be from slow-moving, charged dust. He also points to reports of electrical interference in preflight laboratory tests of the instrument. Instead, O'Brien writes, the signals captured by LEAM occurred when ALSEP turned on or off the electricity for the heaters it needed to survive the lunar nights.

Lewis dismisses that idea. Electrical noise interference was the very first thing the LEAM team considered when the unexpected results came in, he says. And the team corrected for the interference between the lunar instruments found in the laboratory. O'Brien "paints a picture of these heaters cycling on and off and on and off, ... but that's not the way it worked," Lewis says. The heaters turned on only once, at sunset, he says, and remained active through the night.

One of the technicians responsible for building LEAM, Derek Perkins, also rejects O'Brien's analysis. In an e-mail Lewis forwarded to *Science*, Perkins notes the results of



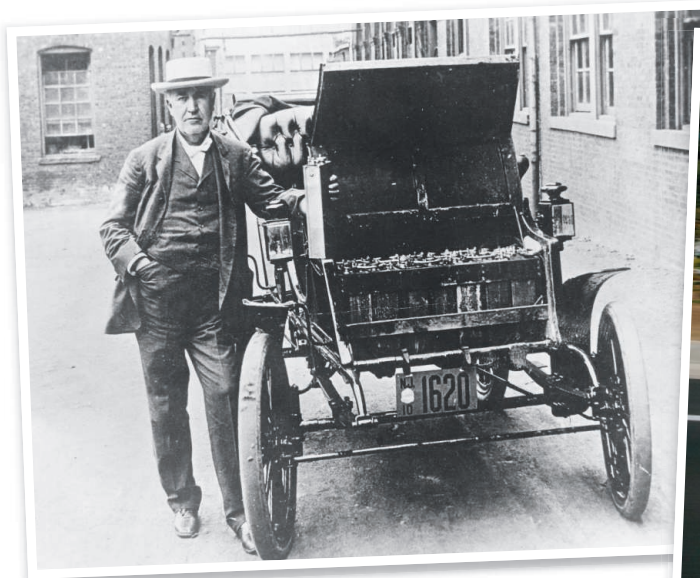
Heater debate. The Apollo 17 LEAM instrument (foreground) was thought to have detected floating dust that could explain the lunar horizon glow (inset), but that may have been interference from heaters on nearby instruments (background).

a simulation using the backup LEAM instrument in 1976. In that study, Perkins simulated firing slow-moving, highly charged particles, similar to the lunar dust hypothesized to be responsible for the horizon glow, at the instrument and found a detection response similar to that of the Apollo 17 experiment.

A conclusive answer to the LEAM debate may never come. The ALSEP data recovery group has located only a few months' worth of LEAM raw data so far. "It's sad that we don't have enough data in hand to really do the analysis," says physicist Mihály Horányi of the University of Colorado, Boulder. Horányi, who, as an editor of the lunar dust special issue of *Planetary and Space Science*, invited O'Brien to submit his review, is the principal investigator on a dust detector set to fly aboard LADEE. That experiment should detect any particles flying about 20 kilometers above the lunar surface. And if it works, lunar researchers may finally decipher what Lovell saw.

—LUCAS LAURSEN

Lucas Laursen is a writer in Zurich, Switzerland.



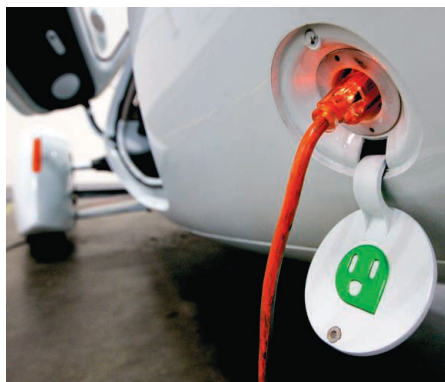
Getting There

Better batteries paved the way for half-decent electric cars. Making improved versions viable for the mass market will depend on a suite of advanced battery technologies now in labs around the globe

RICHLAND, WASHINGTON—Yet-Ming Chiang is at it again. In 2001, Chiang, a materials scientist at the Massachusetts Institute of Technology in Cambridge, came up with a way to dramatically boost the conductivity of a possible new electrode material for lithium-ion batteries. Chiang helped launch a new company called A123 Systems that continues to push ahead with plans to use its improved lithium-ion cells to power millions of electric cars. But A123's batteries, along with all the other lithium-ion cells on the market, are still less than ideal for powering cars. They're expensive and can carry a car only 160 kilometers on a charge. One limitation is that only about half the material in today's lithium-ion batteries actually stores and delivers electricity. The rest is there to control the chemical reactions inside it and ensure that the batteries charge and discharge safely. That's important, of course, but it frustrates battery makers. "I've been saying for years we need to get past that," Chiang says.

Now he's on his way. Last month, Chiang and his colleagues reported in *Advanced Energy Materials* that they've created a new battery design called a semisolid flow cell that's like a battery with a fuel tank. Like today's batteries, the device contains lithium ions that shuttle back and forth either storing or releasing electrical charges on demand. But instead of packaging those

ions along with the electrodes and other apparatus all together, as in a typical battery, Chiang's semisolid flow cell separates the energy-delivery apparatus from energy storage. In this battery, the storage medium is a pair of gooey, black liquids, the consistency of yogurt, that contain nanoscale particles of materials commonly used as anodes and cathodes in lithium-ion cells. These particles are suspended in an electrolyte and separated by a porous membrane. When power is needed, a bolus of each goo is pumped from external tanks into a network of current collectors that extract electrons while lithium ions shuttle through the membrane from the anode particles to the cathode particles.



Plug power. Widespread adoption of electric vehicles hinges on reducing battery costs.

The spent slurries can then be reenergized, as in a normal rechargeable battery, or pumped out and replaced. Other types of flow batteries have been made in the past, but Chiang says the new setup can store up to 30 times as much energy as previous versions. He has launched another company, called 24M, to commercialize the technology.

Chiang's flow cell still faces a host of challenges, such as ensuring that lithium-containing particles in the goo don't settle to the bottom of the battery tank. But Dawson Cagle, a chemist who helps run the batteries program at the Advanced Research Projects Agency-Energy (ARPA-E), says the work is indicative of a new spirit of creativity among battery researchers. "I knew this was different the first time I walked into Yet-Ming Chiang's lab and he was talking about yogurt and toothpaste," Cagle says. "There are so many good ideas out there that there really is a bright future."

If the boldest projects now in labs around the globe succeed, they could provide at least 10 times the energy of today's lithium-ion batteries, enough to make fully battery-powered cars competitive with today's gasoline models. "I have never seen as much interest in batteries as there is now," says Esther Takeuchi, a chemist at the University at Buffalo in New York state. "It's a tremendously exciting time to be involved in energy storage." Mark Verbrugge, who directs the chemical sciences and materials systems lab at GM's Global R&D Center in Warren, Michigan, says carmakers are now "within shouting distance of making battery vehicles for the mass market." And the improvements are adding up. "It's starting to snowball," Verbrugge says.

CREDITS: (TOP LEFT) PHOTO BY GENERAL PHOTOGRAPHIC AGENCY/GETTY IMAGES; (TOP RIGHT AND BOTTOM) PHOTO BY SANDY HUFFAKER/BLOOMBERG VIA GETTY IMAGES

Downloaded from www.sciencemag.org on June 23, 2011

Old idea. From Thomas Edison's 1895 model (left) to today's three-wheeler by Aptera Motors, electric cars have been limited by their batteries.

Balancing act

That's the good news. The bad news is that battery evolution has always been painfully slow. "The progress we have made compared to other technologies is extremely low," says Jürgen Leohold, research chief for Volkswagen Research in Wolfsburg, Germany. It's not for lack of trying. Batteries produce electricity by taking advantage of the fact that some metals hold on to their electrons more tightly than others do, the same principle Alessandro Volta used to make the first battery in 1800. Metals that readily dump electrons are stored at a negatively charged electrode, or anode, while a positively charged electrode, called a cathode, harbors electron-hungry compounds. When a wire or an electric circuit is connected between the two electrodes, electrons stream from the anode to the cathode; at the same time, charged ions travel through an ion-conducting electrolyte to react at the cathode, where they produce a stable compound and complete the electrochemical cycle. If the battery is rechargeable, plugging it into an outlet provides a higher voltage that pushes the chemical reactions in reverse.

Successive generations of battery materials—lead acid batteries, nickel metal hydrides, and now lithium-ion cells—have improved the density of energy that can be stored. But changing battery chemistries raises issues by the bundle. Not only must the new anode and the cathode materials work together, but the ion-conducting electrolyte and membrane separators used in many batteries must also evolve. Problems with any one of these materials can prevent a battery from working, prevent it from recharging, cause it to burn out after a few recharge cycles, interfere with its safety, and on and on. "You have to optimize many different things at the same time," says Venkat Srinivasan, a transportation battery expert at Lawrence Berkeley National Laboratory in California. "It's a hard, hard problem." That's held increases in storage capacity to about 5% per year—a rate that pales in comparison to "Moore's Law" in semiconductors, in which the number of transistors on each chip has doubled every 18 months over a period of decades. And it's given rise to an unwanted rule of thumb that batteries on the market deliver only one-quarter of the energy stor-

age potential of their active ingredients.

Even with that penalty, today's lithium-ion batteries store 120 to 140 watt-hours per kilogram (Wh/kg), enough to give an electric car "a half-way decent performance," Leohold says. In terms of range, that's enough to drive up to about 160 kilometers. "It is acceptable in an urban environment, but it does not allow for long-distance driving," Leohold says. That's why most cars incorporating an electric motor today are gas-electric hybrids. By 2017, Leohold suggests, engineering advances to current lithium-ion cells are expected to boost that capacity to about 200 Wh/kg, or about 240 kilometers. "But to get long-range driving, we need 1000 to 1500 Wh/kg," which Leohold says should boost battery-powered cars close to the range of gas-powered versions today.

The painful question is, at what cost? Most industry estimates place current vehicle battery costs at between \$600 and \$700 per installed kilowatt-hour. For electric vehicles to succeed on the market, the U.S. Department of Energy (DOE) has concluded that batterymakers need to lower that number to \$250 per kilowatt-hour. One recent study by Boston Consulting Group suggested that batterymakers could approach that DOE target by 2020. But others have been more cautious.

A study last year by the National Research Council points out that the usable energy output of batteries is typically lower than advertised. That effectively lowers their range and thus increases their current cost to between \$1250 and \$1700. Costs are expected to decline 35% by 2020, the study says, but that will still leave batteries too costly to spur mass-market adoption of electric vehicles. And cost isn't the only hurdle. Vehicle batteries must be safe and vibration-resistant, must work over a wide range of temperatures, and must be capable of being recharged at least 1000 times while retaining 80% of their storage capacity. "For a less than \$250 a kilowatt-



BATTERY FAQs

Why is lithium so often used in batteries?

Lithium is the lightest metal and has the highest energy density for its weight.

Is there enough lithium to make batteries for millions of cars a year?

In 2005, roughly 21,000 tons of lithium was produced worldwide. More than 6 million tons of lithium reserves are thought to be economically viable to recover. Twice that amount exists in forms not economically viable to recover today. An analysis presented at the meeting* by Paul Albertus of the Robert Bosch Research & Technology Center in Palo Alto, California, suggests there will be plenty of lithium over the near term, through the next 15 years. It's only in the long term, 40 to 50 years from now, that the lithium supply could get tight. Other elements, such as cobalt, could pose a bigger problem, depending on the chemistry of the batteries produced.

How will widespread adoption of electric vehicles affect CO₂ emissions and possible climate change?

It won't, unless the electricity used to power those cars is generated by renewable energy sources. But even if the electricity is produced by coal-fired power plants, many of which exist in rural areas, urban emissions of smog-forming particles could still drop dramatically.

—R.F.S.



hour system, those are very tough goals," says Trygve Burchardt, chief scientific officer of ReVolt Technology, an advanced battery maker in Portland, Oregon.

Even if battery costs drop, electric vehicles are almost certain to cost more than their gas counterparts. So as long as oil is cheap and governments remain uncommitted to limiting carbon dioxide emissions from cars, electric vehicles of all types will face an uphill battle. But most electric vehicle proponents are convinced that rising concerns about energy security, gas prices, and climate change are creating an inexorable demand for better batteries. "The

Online

sciencemag.org

Podcast interview
with author
Robert F. Service.

societal need for energy storage will be much greater in the future than in the past,” says Peter Bruce, a lithium battery pioneer at the University of St. Andrews in the United Kingdom.

Beyond lithium?

Over the past 15 years, lithium-ion battery makers have doubled the energy-storage density of their devices. And battery companies have at least a dozen combinations in the works of new anodes, cathodes, and electrolytes. But to make dramatic strides

dissolved polysulfides, rather than continuing to undergo chemical reactions to form Li_2S —the desired end product when it’s within the cathode—can diffuse back and forth between the electrodes, in the process undergoing reactions that sap the battery of much of its charge-carrying capacity.

Those problems aren’t solved yet. But at the Richland meeting, a couple of groups reported steady progress. For example, Chengdu Liang, a chemist at Oak Ridge National Laboratory in Tennessee, announced that he and his colleagues have

come up with a bromine-based electrolyte additive to fight Li_2S buildup. The additive doesn’t prevent Li_2S from precipitating out of solution, but it reacts with the compound, converting it back into a soluble polysulfide that dissolves into the electrolyte.

Linda Nazar, a chemist at the University of Waterloo in Canada, reported that she and her team had incorporated porous silica particles into their sulfur-carbon cathode. During charging

and discharging, the particles sop up most unwanted polysulfides that form and then release them late in the cycle, when the cell can convert them into solid Li_2S within the cathode. The upshot, Nazar reported, is that the Canadian team’s cathodes have a capacity of about 900 milliamps per gram, a number that in full batteries would likely translate to roughly 600 Wh/kg. “We’re not there yet,” Nazar says of her team’s effort to make LiS batteries practical. “But this is a step along the way.”

As impressive as the energy-storage numbers are for LiS batteries, those that pair lithium with oxygen (also known as lithium-air batteries) make battery experts downright giddy. In theory, their active materials can generate a staggering 12,000 Wh/kg. Lithium-air batteries again use lithium metal as the anode material. But in this case the cathode isn’t sealed in the battery. Rather, oxygen from the air serves as the electron-hungry cathode material. That makes the batteries far lighter—and potentially cheaper—than the competition.

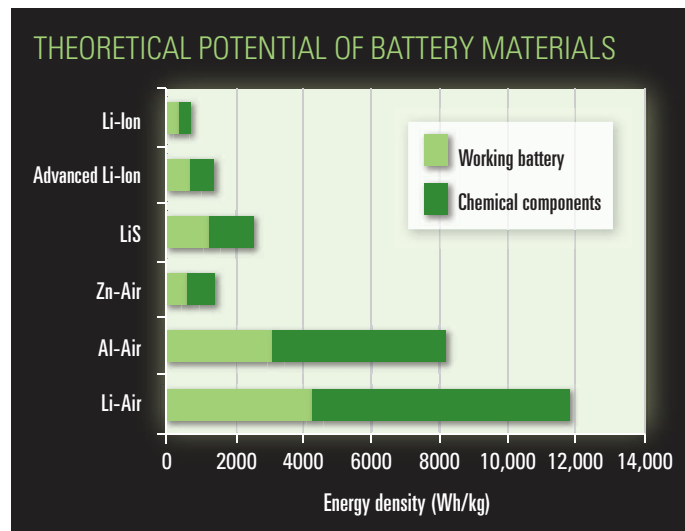
Of course, there are plenty of challenges there, too. Metallic lithium reacts violently with water, which is ever-present in air. It has also been harder to get lithium-air batteries to recharge over large numbers of cycles, as their capacity fades quickly or crashes altogether.

But there has been halting progress on these fronts as well. Over the past several years, researchers at PolyPlus, a battery start-up in Berkeley, California, have made steady progress on creating a solid ceramic electrolyte to coat their lithium metal anode. The ceramic has pores that allow lithium ions to diffuse through, but it blocks water molecules from reaching the anode. Steven Visco, PolyPlus’s chief technology officer, told attendees in Richland that company researchers have built nonrechargeable batteries that work when fully immersed in seawater, with a capacity of 1000 Wh/kg. The hope, Visco says, is that such cells could power undersea robotic vehicles far longer than batteries today can accomplish.

Despite such advances, Visco acknowledges that making fully rechargeable lithium-air batteries continues to prove difficult. Another strategy replaces the solid ceramic electrolyte with a more conventional organic liquid. The capacity of such cells tends to fade quickly, as unwanted chemical reactions within the batteries kill the cells. At the meeting, St. Andrews’s Bruce reported detailed characterization studies that showed that much of the problem arises when byproducts of the lithium-oxygen reactions, such as superoxide and lithium peroxide, react with and break down standard carbon-containing electrolytes. Bruce and others also revealed that alternative electrolytes, such as polyethers and ionic liquids, tend to hold up better. “It’s clear the community is getting a better handle on these systems. But we need quite a bit more research if we’re going to have a chance at getting a practical system,” Bruce says.

Real-world LiS and lithium-air batteries are still likely years away, Srinivasan says, as are other upstarts, such as rechargeable zinc-air and magnesium-based batteries. But Srinivasan adds that even though the challenges of improving batteries almost always appear daunting, decades of persistent, dedicated effort have led to a string of breakthroughs that seem obvious in hindsight. “With [battery] chemistry, you have a lot of knobs you can turn,” Srinivasan says. That’s both good news and bad news, as battery-makers now sift through the vast number of materials combinations for just the right mix.

—ROBERT F. SERVICE



The contenders. Energy-storage materials used in batteries can hold energy at a far higher density (dark green) than working batteries can (light green). When batteries are collected in packs, the storage density drops further.

in energy storage, Srinivasan says, “it’s become obvious to us that we need to start thinking about what’s beyond lithium ion.”

Several such prospects took the spotlight at a meeting* here earlier this month. Among the most tantalizing was a chemical combination known as lithium sulfur (LiS) batteries, which could have a capacity at least several times that of today’s lithium-ion cells. In these batteries, lithium ions shuttle between an anode made from lithium metal and a cathode made from sulfur, typically packed into a carbon-based housing. The basic idea isn’t new, but a host of problems have held back LiS batteries. For starters, when lithium ions move to the cathode they react with solid sulfur, creating a family of polysulfide compounds that can dissolve in the battery’s liquid electrolyte. Some of these, such as Li_2S , readily precipitate out of solution and can coat and clog the electrodes. Equally troubling, the

*Fourth Symposium on Energy Storage: Beyond Lithium Ion, Pacific Northwest National Laboratory, 7–9 June.

BIOTECHNOLOGY

Lab-on-a-Chip Maker Looks to Put Hong Kong on Biotech Map

Better known for business acumen than scientific smarts, Hong Kong is betting on biotech as a new “pillar industry”; a novel biochip suggests it’s on the right track

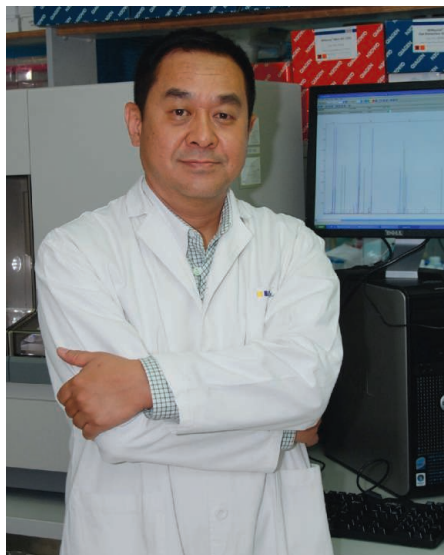
HONG KONG—In 1994, neuroscientist Albert Cheung-Hoi Yu gave up a plum position at Stanford University to join the new Hong Kong University of Science and Technology (HKUST). The career gamble paid off in an unexpected way. In 1997, H5N1 avian influenza hammered Hong Kong. “There was no sensitive tool for diagnosing this virus,” Yu says. Polymerase chain reaction (PCR) tests were not standardized and therefore not reliable, he says. He launched a company to develop molecular diagnostics. As his start-up made headway, Yu aimed higher. He hoped to succeed where larger companies had failed: devise a biochip that quickly diagnoses emerging pathogens.

Overcoming obstacles that dogged previous efforts, Yu’s company, Hai Kang Life, has rolled out a novel chip that binds target DNA strands and coats them with nanoparticles for identification. The Chinese Center for Disease Control and Prevention of Guangdong Province will soon begin testing the chips and automated readers. “It’s a unique system,” says Zhengping Zhuang, a clinical pathologist at the U.S. National Institutes of Health, who is not connected to Hai Kang Life. “You get a readout much faster than with other technologies.”

If Yu’s Electric Field Assisted Diagnostic (EFAD) chip proves its mettle, it will be a milestone in nascent efforts to make Hong Kong a biotechnology hub. For decades, the city reigned as Asia’s financial powerhouse. Then in 2008, the Lehman Brothers collapse sparked a crisis in the financial services industry—and jarred leaders here. “They started thinking about how to diversify the economy,” says Janet Wong, Hong Kong’s Commissioner for Innovation and Technology.

The next year, Hong Kong’s government designated innovation and technology as one of six new “pillar industries.” To stimulate the biotech and pharmaceutical sectors, the government last February unveiled a clutch of measures, including \$138 million for a new medical research fund and a \$5 million initiative to open centers for international clinical trials and translational research.

Success is by no means assured. Hong Kong embraced biotech later than other



Underdog victory. Albert Cheung-Hoi Yu’s young company beat the competition in developing a DNA chip for rapid diagnoses.

regions in China, such as Shanghai, Tianjin, and the new medical city rising in Taizhou. These municipalities have showered companies with tax incentives and spent billions of dollars on infrastructure. Here, meanwhile, land is scarce and expensive. “We never say our cost is low,” Wong says. The city’s chief selling point may be strict enforcement of intellectual-property rights, she says: “We’re attracting IP-sensitive R&D.”

That’s a sea change. When Yu relocated here, he says, “Hong Kong was not a place to do science.” Local universities set out to shift that perception in the 1990s by recruiting overseas talent. During a stint overseeing undergraduate admissions soon after joining HKUST, Yu touted biotech as a potential career option. “But graduates had nowhere to work in Hong Kong. At best they could be a salesperson for a drug company,” Yu says. He realized his own company could play a small role in changing that; Hai Kang Life employs several HKUST grads, including its founding chief operating officer, Terence Lau.

At the outset, Yu’s company picked low-hanging fruit: It was the first private laboratory accredited in Southeast Asia to test foods for genetically modified organisms and to

do prenatal testing for Down syndrome and other chromosomal abnormalities. Then in 2003, the emergence here of the SARS virus spurred Yu to redouble research on a diagnostic lab on a chip. “We saw the bottleneck was hybridization,” he says, in which DNA is dissociated into single strands that bind to a probe. This process typically takes many hours. Yu hit upon a winner: applying an electric field to tweak the capacitance of DNA strands and speed up probe-to-DNA binding. Yu’s team could get DNA or RNA to hybridize in minutes. The technique is also sensitive enough to eliminate the need for PCR to amplify targets. “It’s easy to say you want to make a biochip like this,” Zhuang says. “It’s another thing to do it. Albert is focused and stubborn, and he found a way.”

Another challenge is to identify strands bound to probes. Other companies use fluorescent tags, which require a pricey microscope and trained eyes. Yu’s team floods sample wells with silver nanoparticles that latch onto bound DNA strands. Like a black-and-white picture, the coating’s contrast is picked up by an inexpensive charge-coupled device camera and analyzed by a small automated reader.

The approach is so simple, Yu says, that when Hai Kang Life applied for patents, “we were thinking that someone else must have done this.” They were wrong and now hold 30 patents. It’s no surprise that Hai Kang Life got there first, says Bernard Roizman, a virologist at the University of Chicago in Illinois. Many diagnostics companies prefer to sell large, complicated, and expensive machines, he says. EFAD chip readers are the size of a microwave oven. “Attach it to a power supply, and it’s a lab on a chip on wheels,” Roizman says. “It could identify a new infectious agent in time to curtail its spread.”

When he’s not in the lab, Yu, who moved to Peking University in Beijing in 2002 and now shuttles between Beijing and Hong Kong, promotes biotech here as inaugural chair of the Hong Kong Biotechnology Organization. To better compete with the mainland, Hong Kong’s science park, home to more than 340 companies, has just launched a \$600 million expansion expected to create 4000 R&D jobs. “We need to identify our niches,” for example, nurturing start-ups focused on Chinese medicine or medical devices, says Nicholas Brooke, chair of Hong Kong Science & Technology Parks Corp. “We will be lost if we attempt to be all things to all people.”

The first step is to get an innovative biotech product on the market. Yu’s biochip is poised to do just that. “Nobody in the world would believe that this could be done in Hong Kong,” he says.

—RICHARD STONE

PROFILE: OGOBARA DOUMBO

Mali Researcher Shows How To Reverse Brain Drain

Relying mainly on homegrown talent, Doumbo leads a network in Mali that does state-of-the-art studies of mosquito genetics, tracks drug resistance, and tests new vaccines

BAMAKO, MALI—On a bluff overlooking a flat Sahelian landscape, evening finds most offices empty at the University of Bamako's Faculty of Medicine. But a few lights remain on in the Malaria Research and Training Center (MRTC), and three Ph.D. candidates wait to speak with the director, Ogobara Doumbo. He leaves in a few days for Geneva to present new research affecting World Health Organization (WHO) guidelines on malaria prevention for children. But he makes space in the lab to discuss with a visitor what makes MRTC a paradox.

Doumbo, in his mid-50s but still looking like a student, smiles faintly when he speaks about his protégés, who recently led a roomful of top West African scientists through a comprehensive research discussion. Only in Mali, he says, will you find a critical mass of African Ph.D.s, with no loss to brain drain.

Bamako, a capital city of dusty streets on the banks of the Niger River, is not a place you expect to find a world center for research. Serving one of the world's poorest countries, Mali's health system is stretched to the breaking point. Yet on this bluff known as Point G, with a colonial-era hospital from 1906, Doumbo has built MRTC and nurtured, by his count, five generations of researchers committed to solving one of the continent's most intransigent problems.

Since co-founding MRTC with support from the U.S. National Institutes of Health (NIH) in 1992, Doumbo has led it into state-of-the-art research on mosquito genetics, vaccine testing, and drug resistance. Furthermore, MRTC supports a network of research-affiliated clinics throughout Mali in very basic village settings. Doumbo has cultivated this cadre for 15 years, what he calls "the bush doctor initiative," to bring top-quality medical research and practice to villages. The conditions would seem ripe for an exodus of highly trained physicians. In most developing countries, simply keeping capable scientists in the capital is difficult. A 2007 World Bank study noted the accelerated migration of skilled professionals, particularly in medicine, and its important effects on poor countries. MRTC has found another path.



Traditionalist. A grandson of healers, Doumbo dreamed of becoming a village doctor.

"Quite often many senior researchers who could mentor the younger ones have themselves left," says Wilfred Mbacham, executive director of the Multilateral Initiative on Malaria, based in Yaoundé, Cameroon. Those who aren't lured to higher-paying international jobs get tapped for political appointments away from the university, he adds. A country's political climate—and its valuation of research—are contributing factors. Mbacham has known Doumbo since 2003 and says that Doumbo saw the need to create a stable environment. "Very early on, he set up a grant-administration program that was attractive for more funding," Mbacham says.

Mali has many problems shared by other sub-Saharan countries, including minimal infrastructure and corruption. In 2010, the Global Fund to Fight AIDS, Tuberculosis and Malaria suspended its malaria programs in Mali after an internal report found health department officials (not MRTC) had siphoned program funds.

Nor has MRTC been immune to charges that it benefited from donor favoritism. In

the early years, says Stephanie James, director of science at the Foundation for the National Institutes of Health, a public charity in Bethesda, Maryland, "I know there were some jealousies in the university." And some predicted that Doumbo "would never relinquish control over projects," recalls Christopher Plowe, a researcher at the University of Maryland School of Medicine in Baltimore and an MRTC collaborator. "Last year I was struck by how wrong that prediction was."

Path to parasites

Doumbo, a son and grandson of traditional healers, grew up in a Dogon village 965 kilometers northeast of the capital. He first rode in a car as a teenager in 1971, to take his secondary-school certification exam in the town of Bandiagara. He never intended to go into research. "I really wanted to be a doctor and to serve in the bush," he says.

After obtaining an M.D. degree at the University of Bamako and finishing a residency in internal medicine at Point G, Doumbo began to practice in 1981 at a clinic at Selingué, about 2½ hours south of the capital. There he aimed to win over local skeptics of Western medicine. The many C-section deliveries he performed were dramatic proof that his methods could save lives. "He was famous for being the guy who handled complicated obstetric labor emergencies and surgeries," Plowe says.

In Selingué, Doumbo found larger problems: river blindness, schistosomiasis, and malaria. "I saw a lot of people suffering," he says. He realized he could have greater impact by recruiting more young doctors to help. He returned to his studies, earning an M.Sc. in tropical medicine from the University of Marseille studying under parasitologist Philippe Ranque and a Ph.D. in parasitology from the University of Montpellier.

Doumbo also saw a role for indigenous medicine. Pragmatically, he saw traditional healers as scarce health care providers already treating rural dwellers, often with useful local knowledge, and thought it better to gain them as partners. "The best way to promote traditional medicine is to show that both types of medicine can work together to resolve a public health problem. This is what we are doing with malaria."

Choosing partners

Harold Varmus, Nobel laureate and now director of the U.S. National Cancer Institute, visited Mali when he was NIH director in 1997 and traveled to several remote villages. "Doumbo and his senior colleagues grew up in villages without electricity, worked hard

for an education in Mali and France, and decided to build a scientific effort in Mali to combat one of its most difficult diseases," Varmus says. "His determination, deep knowledge of malaria, and positive effects on co-workers and government leaders were quickly evident, even in a short visit.

"One of the great things about this effort was his engagement of villagers in the projects," Varmus recalls. That yielded a better understanding among villagers of how transmission of the malaria parasite *Plasmodium* takes place. After that, they built "multi-functional" health clinics and wells for clean drinking water. Doumbo aims for "a new way of thinking," he says, "of how we can deal with rural areas," one that involves them as partners.

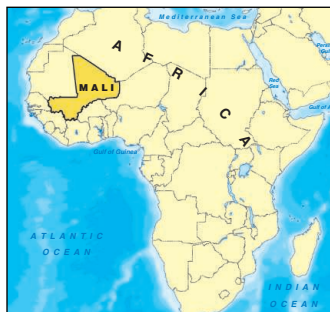
Since 1997, Karamako Nimaga has been a researcher-clinician in Marka COUNGO, a village 2 hours east of the capital. His clinic there collected data for a paper he co-authored with MRTC colleagues chronicling the failure of chloroquine, then the standard antimalarial drug. The findings helped overturn WHO and national policies on malaria prevention: WHO changed its guideline in 2006 from chloroquine to artemisinin-based combination therapy as the main drug treatment. MRTC has also pioneered new treatments for prevention with pregnant women and children and won international awards.

Doumbo's style has sometimes raised hackles, however. Health officials argue that the government's rural clinics, which are village-funded, get overshadowed by MRTC's network of international-funded clinics like Nimaga's with lab facilities. Tensions came to the fore when MRTC recommended that the health department abandon chloroquine, in line with WHO's policy. Doumbo and his staff spent a day explaining to ministry staff the WHO recommendation and supporting data. Some officials, seeking to keep chloroquine, focused their frustration on Doumbo. "As you can imagine, a lot of people are reluctant to make any changes," Doumbo says of that battle. But finally, WHO's policy was adopted.

Bernhards Ogotu, senior scientist with the international INDEPTH Network, who coordinates malaria research from Ghana, notes that MRTC has made remarkable

progress for the same reason that makes it controversial: MRTC has "marketed the research agenda to the country's political leadership," getting leaders "to appreciate the importance of research and its long-term impact on Mali."

Doumbo has mastered the metaphors that get politicians' attention. He compares malaria's toll to three tsunamis every year and says: "Africa has lost a lot of Einsteins, a lot of Pasteurs, a lot of Bill Gateses because of malaria. And if you're able to eliminate malaria, you will see it increase the general creativity in a country and the



Home base. The Malaria Research and Training Center in Bamako has fostered, by Doumbo's count, five generations of researchers. Many work today in rural clinics.

ability of people to innovate and bring science to make their own solutions." This encourages younger scientists, Ogotu says: "They can see that science is valued, up to the highest office."

The next generation

Doumbo has shown a knack for nurturing younger scientists. Leaders need to create capable successors. "If this is not done, then

scientists will burn out and exit," Ogotu says by e-mail. Mbacham agrees: Doumbo has given responsibility to those Mbacham calls "generation F2, who now begin to have their own teams and success stories."

One star of the younger generation is Abdoulaye Djimé, who started at MRTC in 1993 after putting himself through the University of Bamako's Pharm.D. program. Djimé was managing a pharmacy in the mornings and volunteering at MRTC in the afternoons, Plowe recalls. Doumbo assigned the young pharmacist to learn a technique for identifying gene mutations and later, with Plowe, arranged for Djimé to attend short-term training at NIH. Typically, a director would feel obligated to send a more senior technician, Plowe says, but Doumbo made an exception "for somebody who he thinks has that spark that's going to lead to success."

Plowe and Doumbo helped Djimé get into a Ph.D. program at the University of Maryland, Baltimore. He returned to Mali in 1999 with a research plan and prospects for a grant. "That collaboration," Plowe says, "led to a lot of good work published over the years." Djimé heads MRTC's drug resistance unit and is the first person from West Africa to receive a Howard Hughes grant.

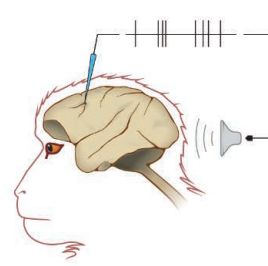
"It really is a meritocracy," Plowe says, "which I think is unusual."

As night falls with his Ph.D. students waiting, Doumbo describes the key factors for keeping talent: careful selection of staff from among the medical students, a mentor for each graduate student going abroad, and workshops on subjects such as grant-writing so they can find funding for research on their return. "Maliens don't like leaving their country," Doumbo says. "I never even ask them to come back. Never. I say, 'It's up to you if you want to join the team and help the population.' But they all come back." Varmus says the strategy works well, in part "because the students have a good place to which to return to do research."

Doumbo's emphasis on self-sufficiency, stemming from his own background in a remote village, may be a wave of the future. "I think we're in a period in Africa where you can no longer centralize," he says. "We have no choice but to go toward decentralization for all activities, to give responsibility to people." In community-based medicine, people find their own solutions. "That's why I'm confident that will be the future of Mali and the future of Africa."

—DAVID A. TAYLOR

David A. Taylor traveled to Mali with support from the International Reporting Project.



LETTERS

edited by Jennifer Sills

Dolphin Research:
Continue Captivity

I WAS DISAPPOINTED BY THE NEWS FOCUS STORY “ARE DOLPHINS TOO smart for captivity?” (D. Grimm, 29 April, p. 526), which questioned the importance of dolphin research in zoological parks and aquariums.

The Alliance of Marine Mammal Parks and Aquariums agrees with the scientists in the article: Continued research with dolphins is necessary to advance the scientific community’s understanding of dolphin cognition as well as physiology and reproductive biology. This research cannot be replicated in the wild and is essential to protecting dolphins in their ocean environments. In advocating the animal rights’ agenda, Lori Marino goes overboard, to the detriment of good science.

The “new paradigm” of “collaborative research,” which would limit all dolphin and whale research to those animals in the wild that “have ‘decided’ to interact with humans,” would likely be prohibited in the United States under the Marine Mammal Protection Act (1)



because it could change natural behaviors, harming the animals. The U.S. National Marine Fisheries Service has long promoted its Protect Dolphins campaign (2) to educate the public about the harm caused by interacting with marine mammals in the wild.

Marino is also wrong on dolphin life spans. A comparison of their life spans in Alliance member facilities and peer-reviewed, published papers on animals in the wild confirms that dolphins in U.S. parks and aquariums live almost twice as long as those in our oceans and bays (3–5). It’s no wonder; these animals get state-of-the-art medical care, are well fed, and have no worries about becoming prey—as one scientist points out in the article.

Science asked anthropomorphically if dolphins are “really unhappy in captivity.” Rest assured that scientific, quantifiable measures—reproductive success, life spans, and cortisol and aldosterone testing showing lack of stress—indicate they are very happy.

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Dolphin Research:
Educating the Public

I FEEL COMPELLED TO RESPOND TO THE NEWS Focus story “Are dolphins too smart for captivity?” (D. Grimm, 29 April, p. 526). I conducted the study showing mirror self-recognition in dolphins, and Lori Marino and I coauthored the paper that appeared in *Proceedings of the National Academy of Sciences* in 2001 (1). The News Focus story misleadingly suggests that scientists such as myself support keeping dolphins in captivity for the purely selfish purpose of research. Nothing could be further from the truth. Along with other researchers in this field, I am opposed to dolphins being taken from the wild, and I have insisted that any facility

keeping dolphins do so with the utmost concern for their wellbeing.

Marino and I differ on whether it is possible to treat dolphins humanely in captivity, and we differ on our priorities for improving dolphin welfare: I believe that the more we learn about them and the more people can get to know them in their rich complexity, the greater the chance to rally world opinion to stop the drive-hunt slaughter that continues every year. I also believe that we must do everything possible to stop the drive hunts, including the humane educational practices of high-quality aquariums. I applaud *Science* for providing the data required to turn science into policy that protects these animals from pain and suffering.

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Effects of Creating Order
from Chaos

IN THEIR ANALYSIS OF DISORDER AND DISCRIMINATION (“Coping with chaos: How disordered contexts promote stereotyping and discrimination,” Reports, 8 April, p. 251), D. A. Stapel and S. Lindenberg find that a disordered environment promotes more discrimination than one that is clean and ordered.

Unfortunately, their design precludes this conclusion. Part of the study involved

subjects (all Caucasian) choosing one seat among six, with the first seat occupied by a confederate (either a white person or a black person). In the ordered condition, the distance between the seat chosen and the confederate was unaffected by the race of the confederate, whereas in the disordered condition, the distance was greater if the confederate was black than if he was white.

Although the authors were careful to balance the sexes of the subjects (half female, half male), they did not balance the races of the subjects (all Caucasian). Their conclusion, that disorder affects stereotyping, only holds if results from the neglected populations (e.g., blacks) are irrelevant.

The unexamined conditions are not irrelevant. Suppose blacks sat farther from blacks than whites under the disordered condition (just as the whites did). This would bring the entire conclusion into question. Suppose they sat farther from whites than blacks in the disordered condition. This would support the conclusion, but show that stereotyping is not a white-only phenomenon. Suppose they sat farther from whites in the ordered condition than in the disordered condition. This would

suggest disorder increases stereotyping by whites that and that order increases stereotyping by blacks, and therefore vitiate their policy recommendations: "One way to fight unwanted stereotyping and discrimination is to diagnose environmental disorder early and to intervene immediately by cleaning up and creating physical order" (p. 253).

WILLIAM VAUGHAN

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Response

VAUGHAN CLAIMS THAT WE SHOULD HAVE also investigated stereotyping by blacks. Of course, this is a possibility, but the fact that we researched whites in no way precludes our conclusion that physical disorder increases stereotyping. First, we did not define stereotyping by how far people sit away from members of a different race (that is, behavior based on the content of stereotypes). We identified stereotyping itself by the scores on attributing traits to groups (in our case, Muslims, homosexuals, and the Dutch). Second, we know of no evidence that would suggest that stereotyping is limited to Caucasians. There is also

no evidence to suggest that only Caucasians have a need for order. There is thus no obvious need to counterbalance our study with conditions in which we test the effects for different races. Not repeating experiments explicitly with various races is based on the informed presumption that basic psychological processes pertain to human beings rather than to members of a certain race.

It is likely that deviations from normative expectations (i.e., "the way it should be") increase the need for order and thus also the prevalence of stereotyping and discrimination; there could be cultural, but not purely racial, differences with regard to these expectations. With regard to the effect of physi-

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

cal disorder, even the cultural differences are likely to play a minimal role. Certainly in industrialized societies, physical disorder may be tolerated to various degrees by different subcultures, but in the public realm, it very likely deviates from “the way it should be” for just about everybody.

In our opinion, the policy recommendation “to diagnose environmental disorder early and to intervene immediately by cleaning up and creating physical order” is and remains fully justified by our results.

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Science Festivals Open Doors

IN THEIR EDITORIAL “CELEBRATING THE CULTURE of science” (11 March, p. 1242), J. Durant and A. Ibrahim call on the scientific community

to get involved in supporting the proliferation of science festivals. Many festivals exist—the European Science Events Association lists nearly 100 of them (1)—but there are many cities without any. We agree with the authors that sharing scientific culture, in particular an understanding of the research process, allows people to experience “values of reasoning, openness, tolerance, and respect for evidence.” Thus, we suggest that the scientific community target countries facing important social challenges and where young people are calling for empowerment.

As university students, we created an annual science festival, called Paris Montagne (2), as a direct response to the 2005 riots in French suburbs. More recently, we worked with university students to initiate scientific workshops to bring together Serb and Croat children in the still tense and segregated city of Vukovar, Croatia (3, 4). In 2010, we organized the first Palestinian science festival, which took place in five Palestinian cities, including Gaza (4). Most important, all these actions have been done through a bottom-up approach, involving first, students, Ph.D. candidates, postdocs, and young researchers;

and only later the support of Nobel Prize or Fields Medal winners.

Empowerment through the discovery of scientific research also allows the local facilitators of the festivals to remain more neutral than if they were involved in direct civic education or political action.

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CORRECTIONS AND CLARIFICATIONS

Editors’ Choice: “Nitrogenase’s nascence” by N. S. Wigginton (20 May, p. 896). The text should have read that molybdenum (Mo)-dependent nitrogenases “originated later in time than anticipated” rather than “originated after vanadium- and purely iron-based variants.”

PHYSICS

Let's Be Fysiksists Again

Matthew Wisnioski

In 1968, a cartoon hippie strumming on a slide rule admonished potential employees of the United Nuclear Company to “drop in”:

If you despise routine and regimentation, we're more than halfway interested in you. If you're skilled in any one of many scientific or engineering disciplines, we want you. We need freethinking creative types to help us design and develop new processes and equipment for the future of the nuclear industry.

If you're our man, you'll find $E=MC^2$ more mind-expanding than LSD. You'll find suburban Westchester more comfortable than Hashbury or Tompkins Square. And you'll find our generous benefits program more nourishing than hippy stew. (1)

In this potted recruitment joke were warnings of what historian and quantum physicist David Kaiser calls a “perfect storm.”

After four decades of scholarship documenting how the Cold War changed American science, the scale of the transformation continues to shock. No discipline was more entangled than physics. Driven by federal manpower demands, the production of Ph.D.'s increased more than 20-fold between 1945 and its peak in 1971. Enrollment in individual graduate courses at Berkeley and MIT regularly exceeded 100 students. The practical and ideological constraints of expansion played a role in evaluating what topics were worthy of inquiry. Armed with Feynman diagrams, postdocs were told to “shut up and calculate,” while *Physical Review* instructed referees to reject any paper on quantum mechanical interpretation that failed to make quantitative predictions. In this culture of “hyperpragmatism,” John S. Bell's 1964 proof of nonlocality (2)—retrospectively named “the most successful scientific theory of all time” (3)—languished for years without citation.

By the time United Nuclear gave its pitch, however, physics looked less like the endless frontier than a real-estate bubble about to pop. In *How the Hippies Saved Physics*, Kaiser shows that to a growing number of students,

faculty, and industrial scientists, suburban comfort no longer was a fair trade for routine and regimentation. Congress revoked the exemption that made graduate school a refuge from the military draft. The Department of Defense reconsidered its return on investment in basic science. Then there were the hippies, who many in the scientific community believed were a threat to the very foundation of Western rationality. Physics was besieged on multiple fronts, and the demand for physicists plummeted. For nearly two decades, until the late 1960s, the placement office of the American Institute of Physics had listed more jobs than graduates, but in 1971, there were 1053 applicants for 53 positions.

Kaiser chronicles life after the collapse. In 1975, Elizabeth Rauscher and George Weissmann, two Berkeley graduate students with nothing left to lose, created the Fundamental



“The ‘new physicists’ as countercultural darlings.” With their interests in consciousness, mysticism, and the paranormal, members of the Fundamental Fysiks Group—here (standing, left to right) Jack Sarfatti, Saul-Paul Sirag, Nick Herbert, and (in front) Fred Alan Wolf—garnered considerable attention from the media. (Photograph circa 1975)

How the Hippies Saved Physics
Science, Counterculture,
and the Quantum Revival
by David Kaiser
Norton, New York, 2011.
400 pp. \$26.95, C\$31.
ISBN 9780393076363.

Fysiks Group to study the “spooky actions at a distance” of quantum reality. Their informal seminar linked a network of underemployed physicists, Eastern mystics, and spoon-bending psychics funded by a fried chicken

magnate, *est*-guru Werner Erhard, and the Central Intelligence Agency. Given that chemical substances could alter human consciousness and that quantum information appeared to travel faster than light, the group asked, why could not cognitive manipulation of matter be possible? Participants conducted

experiments on extrasensory perception and built a “metaphase typewriter” out of a mainframe computer, radioactive thallium, a Geiger counter, and a teletype machine—which they used in an attempt to channel the spirit of Harry Houdini.

The Fundamental Fysiks Group appears as the ultimate test case of the boundary between science and pseudoscience. Indeed, scholars of science studies in the 1970s and early 1980s constructed their theories of demarcation with reference to practitioners in its network. Drawing on extensive interviews, citation index statistics, and lucid technical explanations, Kaiser shows the inherent plasticity of mainstream and fringe. Scientists who blended physics with parapsychology (psi) received support from leaders of the profession, including Geoffrey Chew, Victor Weisskopf, and John Wheeler, with the latter's notion of a “participatory universe” serving as a foundational inspiration. While they might have rejected psi phenomena (e.g., extrasensory perception, psychokinesis, telepathy) out of hand, even hardheaded Nobel Prize winners were not above a nude steam bath at the famed Esalen workshops on quantum physics and the nature of reality. The margins also yielded results: Fritjof Capra's *Tao of Physics* (4)—which treated quantum physics and Eastern mysticism symmetrically—became a staple text of physics pedagogy. Group member Saul-Paul Sirag, a college dropout, published two articles in *Nature* (5, 6) while working as a night watchman.

For Kaiser, the Fundamental Fysiks Group offers a morality tale about modes “of doing physics and of being a physicist.” The scientists

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behind the first quantum revolution—Niels Bohr, Albert Einstein, Werner Heisenberg, Erwin Schrödinger—worked in an “earnest” and “informal” “tight-knit community,” quoted Hindu scripture, dabbled in Jungian depth psychology, and chose the yin-yang symbol for their coat of arms. World War II and its resultant organizational culture ended the “golden age.” To a person, members of the Fundamental Fysiks Group, who were trained in the new regime, described a childhood love of science dashed by “turn-the-crank stuff.” Down and out in Northern California, they resurrected the “big-picture search for meaning.”

Using an analogy to Ireland’s medieval monks, Kaiser shows how the interpretive work of hippie physicists “saved” Bell’s theorem. John Clauser, a postdoc at the Lawrence Berkeley Laboratory, published the first experimental verification that the spin of subatomic particles correlated across distances that could not be explained by local forces (7). That did little to improve his job

prospects, but it introduced him to Rauscher and the group. A decade later, Nick Herbert, the mastermind behind the metaphase typewriter, seemed to achieve a viable model of superluminal communication (8). He conceived of a means to send messages instantly across any distance by measuring the polarization of individual photons, cloned by laser amplification to overcome the uncertainty principle. Though flawed, his thought experiment was the catalyst for Bill Wootters and Wojciech Zurek’s proof that an arbitrary unknown quantum state cannot be copied, a discovery that made possible the billion-dollar quantum cryptography industry (9).

An argument premised on the Middle Ages suggests a new era of light. As historians turn to projects like quantum cryptography with the same energy once reserved for the Cold War, competing interpretations hold court. In the “commercialized science” camp, Philip Mirowski describes a dangerous creep of market forces into knowledge production, characterized by managerial innovations such

as the contract research organization (10). Steven Shapin, in contrast, argues for continuity in the modes of doing science and being a scientist, offering a full-throated defense of research managers and venture capitalists (11). In his thick description of the Fundamental Fysiks Group, Kaiser mostly avoids a position on the contours of late-20th-century technoscience. The career of Jack Sarfatti, the group’s mercurial network builder, gives credence to the rise of a privatized, entrepreneurial mode in which the mantra “we are all one” spawns unbreakable bank transfers. Once a bohemian critic of the military-industrial complex, Sarfatti now describes himself as a “counter cultural radical conservative who hob nobs with Reaganites [and] billionaires.” At the same time, Kaiser argues, freewheeling online communities like the FQXi (the Foundational Questions Institute) maintain a necessary dialectic between the wild borders and the tweedy core of the scientific enterprise.

Though intended for the scientifically literate general reader, it is hard not to interpret Kaiser’s praise for the quantum revival as a warning in humanism’s present storm. Confronted with an anemic job market in a system that has embraced the commercialization ethic, one professor has issued a national warning to prospective graduate students to “Just don’t go” (12). Meanwhile, the president of the American Historical Association defends the profession against charges of esotericism, jargon, and politicization (13). Kaiser forgoes eulogy or righteous battle and instead emulates Capra in an homage to the fundamental strangeness of the past. Meticulously researched and unapologetically romantic, *How the Hippies Saved Physics* makes the history of science fun again.

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BROWSINGS

The Quantum Story: A History in 40 Moments. Jim Baggott. Oxford University Press, Oxford, 2011. 505 pp., illus. \$29.95, £16.99. ISBN 9780199566846.

Richard Feynman famously claimed that nobody understands quantum theory. Nonetheless, it may be “the most successful theory of physics ever devised.” Through descriptions of key moments, science writer Baggott charts the development and consequences of quantum ideas. These 40 episodes range from Max Planck’s December 1900 discovery of his “quantum of action” in black-body radiation to the “crisis” of superstring theory. In an optimistic epilogue, Baggott sees hope for the future in current particle-physics research—whether results from CERN’s Large Hadron Collider simply confirm the existence of the Higgs boson or overthrow current quantum field theory. Regardless, he has provided an accessible and informative history of physics “so profoundly at odds with a common-sense conception of the world.”

Guidebook for the Scientific Traveler: Visiting Physics and Chemistry Sites Across America.

Duane S. Nickell. Rutgers University Press, New Brunswick, NJ, 2010. 275 pp. Paper, \$21.95, £14.95. ISBN 9780813547305.

Both armchair travelers and those who wish to make science-focused stops during their journeys will find much of interest in Nickell’s short accounts. In addition to covering several science museums, he introduces a wide-ranging selection of locations associated with famous physicists and chemists; government, academic, and industrial research labs; and sites that highlight particle physics, nuclear weapons, or energy.

Long for This World: The Strange Science of Immortality. Jonathan Weiner. Ecco, New York, 2010. 320 pp. \$27.99, £17.29. ISBN 9780060765361. Paper, 2011. \$15.99, £9.88. ISBN 978-0060765392.

Gerontologist Aubrey de Grey, the book’s central figure, believes that science will soon be able to radically extend the expected human life span and that doing so will bring innumerable benefits. Many of the other researchers whose stories and research Weiner weaves into this account of the science and pseudoscience of immortality doubt both the likelihood and desirability of our forestalling death. The author writes engagingly on such topics as the evolution of aging, free-radical damage of mitochondria, Methuselah mutants that live much longer than other members of their species, and the Sir2 gene that may underlie some effects of extreme calorie-restricted diets. His thoughtful philosophical reflections demonstrate that he did not succumb to de Grey’s proselytizing, as he concludes, “The trouble with immortality is endless.”

Mathematics Teachers' Subtle, Complex Disciplinary Knowledge

Brent Davis

What mathematical competencies must a teacher have to teach the subject well? This has proven difficult to investigate (1). A current view is that teachers' knowledge of mathematics "remains inert in the classroom unless accompanied by a rich repertoire of mathematical knowledge and skills relating directly to the curriculum, instruction, and student learning" (2). Unfortunately, there is no consensus on which "knowledge and skills" might activate teachers' inert knowledge. Two perspectives prevail, neither with a research base that enables strong claims about practice. The majority of current studies focus on explicit knowledge of curriculum content and instructional strategies. Such knowledge might be assessed directly through observation, interview, or written test (2), with a parallel research emphasis on the formal contents of teacher education programs [e.g., (3)]. A second school of thought, presented here, is that the most important competencies tend to be tacit, like skills involved in playing concert piano, learned but not necessarily available to consciousness.

Tacit Knowledge

Teachers' tacit knowledge includes many instantiations invoked to introduce and elaborate concepts, e.g., analogies, metaphors, and applications. Such instantiations are important in early mathematics learning. Teachers in high-performing jurisdictions, such as Hong Kong and Japan, were roughly twice as likely as U.S. teachers to invoke varied interpretations of concepts (4). Analogies can be useful, provided novices have access to sustained interpretive assistance (5, 6). Despite considerable research on instantiations of grade-school mathematics concepts (7, 8), the topic has not been systematically incorporated into teacher preparation. This gap raises interesting issues, which can be highlighted through popular understandings of multiplication.

If you were to ask English-speaking students, parents, or teachers to define multiplication, you would likely hear a regular pat-

tern of response. Most will insist that multiplication is repeated addition and/or a grouping process. This definition works for natural numbers, but it begins to break down as early as the middle grades. How, for example, does one add $\frac{5}{6}$ to itself $\frac{3}{4}$ times, d to itself π times, or -2 to itself -3 times?

Notably, the importance of alternative interpretations has not been lost on the authors of classroom resources. Grade-school textbooks used in the English-speaking world typically invoke about a dozen distinct instantiations of multiplication by eighth grade: e.g., stretching and compressing a number line, array making, area generation, and a linear function. However, it is not clear that such text-based exposure translates into deliberate in-class examination of diverse interpretations and their entailments. Lacking such attention, learners may miss opportunities to develop robust and flexible understandings.

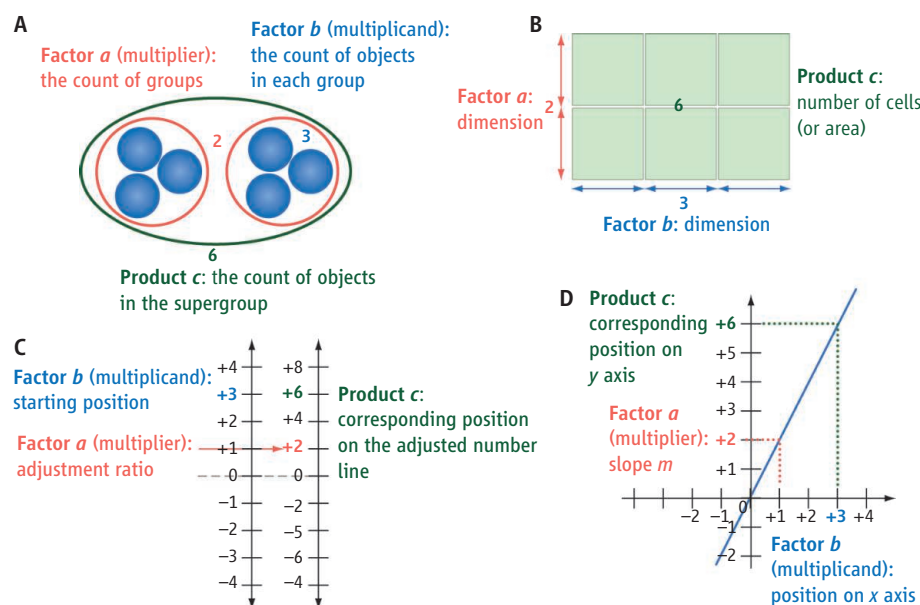
Learning Mathematics

The issue is not with the concept of multiplication itself. Within formal mathematics, multiplication is logically consistent and well defined, albeit the definition continues to evolve with the emergence of new num-

Development of tacit skills, such as use of analogies and metaphors, should receive more attention in preparing math teachers.

ber systems and other conceptual developments (9, 10). Nor is the concern with multiplication in particular. The same argument for diverse instantiations can be made for addition, number, equality, and so on. For educators, the issue is more the dynamics of learning mathematics than the structures of formal mathematics. Young learners' understandings are anchored to narrow, idiosyncratic bands of experience and interpretation. How might they be helped to make sense of unfamiliar situations in ways that are mathematically sufficient but neither overly rigid nor overwhelmingly complex?

This question can be difficult to answer for the expert, who moves effortlessly among instantiations, making choices fitted to the subtleties of the situation (11). To illustrate, the figure shows four different interpretations of multiplication. Although there are some overlaps, moving among these four involves some conceptual leaps. It is not simply that the images are different; the actions that are mapped onto the concept of multiplying (clustering versus array making versus compressing versus sloping) are experientially distinct. Different mappings open up and shut down different interpretive possibilities (12).



" 2×3 " as viewed through four interpretations of multiplication. (A) repeated grouping, (B) grid (or area) making, (C) number-line stretching or compressing, and (D) a linear function ($y = mx$).

Collected together, they offer complex possibilities not present in any single instantiation (13).

The expert's ease of selecting and blending interpretations helps mask the subtle complexity of the knowledge. This subtle complexity makes it difficult to research the nature, extent, and relevance of teachers' tacit disciplinary knowledge. Investigations into the relation between teachers' formal mathematical preparation and their effectiveness have regularly shown little or no correlation between courses in formal mathematics taken by teachers and the performance of their students on standardized tests (14). This has troubled mathematics educators for decades, but from the perspective of tacit knowledge, there seems little reason to expect a strong relation. Teachers' university courses in mathematics typically focus on completed ideas, wrung free of the messiness and complication involved in coming to a new insight. Teaching young learners, in contrast, is largely about drawing logical consistency from diverse instantiations. Humans are not principally logical creatures, but analogical, our capacity for logic reliant on the tendency to make connections (15).

This emphasis on associative learning highlights a distinction between an expert's knowledge and a teacher's knowledge. Whereas it is the mathematician's task to pack insights into tight formulations (theorems, formulae, and so on), it is the teacher's task to unpack (16). This insight led to the notion of "profound understanding of fundamental mathematics" to describe the necessary knowledge for effective teaching (17).

The descriptor "fundamental" may be antithetical to researching the complexity of teacher knowledge. It suggests primary principles—basic building blocks—that can be identified, cataloged, transmitted, and tested. As the example of multiplication shows, it is not clear that instantiations of concepts operate as fundamentals. They appear more to work as agents in an ever-evolving system. It may be more productive to think in terms of "profound understanding of emergent mathematics," the knowledge needed by teachers more than a well-cataloged set of basics. As with any domain of profound human competence, most of it is necessarily tacit (18). It is unlikely that an individual could be consciously aware of the ranges of interpretations that might be invoked for the broad array of concepts covered in school mathematics.

Thus, teachers' mathematics might be more productively viewed as a learnable disposition rather than an explicit body of knowledge. Teachers' attitudes toward exa-

vation and creation of instantiations may be as important as their backgrounds in formal mathematics. Such a disposition is learnable to some extent—by, for example, involving teachers in identifying useful instantiations, investigating their utility, combining them into more powerful interpretations, and using new insights to inform practice (19). Although still in early stages, research indicates that focusing on usually tacit knowledge can have immediate, significant, and sustained impacts on teachers' knowledge of mathematics, perspectives on learning, and classroom practices, as well as student engagement, understandings, and attitudes (13).

Research into tacit knowledge is also difficult in that this focus conflicts with deeply entrenched beliefs about mathematics and learning. For centuries, school curricula have been developed around the assumption that human learning is a principally logical process (20), contributing to programs of study that consist of parsed and sequenced concepts. Directed linear movement through series of concepts may be incompatible with the goal of deep understanding, given the complex, evolving, networked structures of personal understandings of number, equality, addition, multiplication, and so on (11, 15).

Assumptions about fundamentals and linear progress have supported an approach to curriculum and testing that contributes to (and perhaps relies on) narrow, rigid definitions. Among alternatives is a conception that invokes an evolving network (versus rigid hierarchy) image for a knowledge domain. This shifts emphases to seeking out associations and crafting elaborations (e.g., "How can we think about multiplication here?"). A network approach might be facilitated by new media technologies that enable, e.g., hyperlinks, ongoing revision, and collective processing. Attending to inherent complexities of mathematics will require different curriculum structures and teaching practices than those that prevail in public schools.

New structures and practices might help improve attitudes toward the discipline. Currently, in the middle grades, there are proliferations of meaning for many already-defined concepts, e.g., addition, multiplication, number, and equality. Perhaps these "explosions" of meanings, coupled to increasingly abstract applications, might contribute to the common cocktail party confessions, "I was good at math until grade 6" or, more troubling, "I liked math until grade 6." A learner trying to understand, faced with sudden complication and little interpretive assistance, might begin to dislike the subject matter. The alternatives to deep comprehension—rote memorization

and routinized application—make for neither an engaging mathematics (21) nor one well suited to emerging needs.

On that point, approaches to how teacher knowledge is studied appear to be coevolving with perspectives on why mathematics is taught in the first place. Schools have long emphasized the development of technical competence, which was an obvious need in an industrial economy. In a knowledge-based economy, the development of conceptual fluency is of increased importance and has been the focus of major initiatives in school mathematics (22). Emerging research into the subtlety and complexity of teachers' knowledge not only reveals that these initiatives have fallen far short of their lofty goals, it may offer an important route to achieving them.

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Synthetic Physiology

Brian Y. Chow and Edward S. Boyden

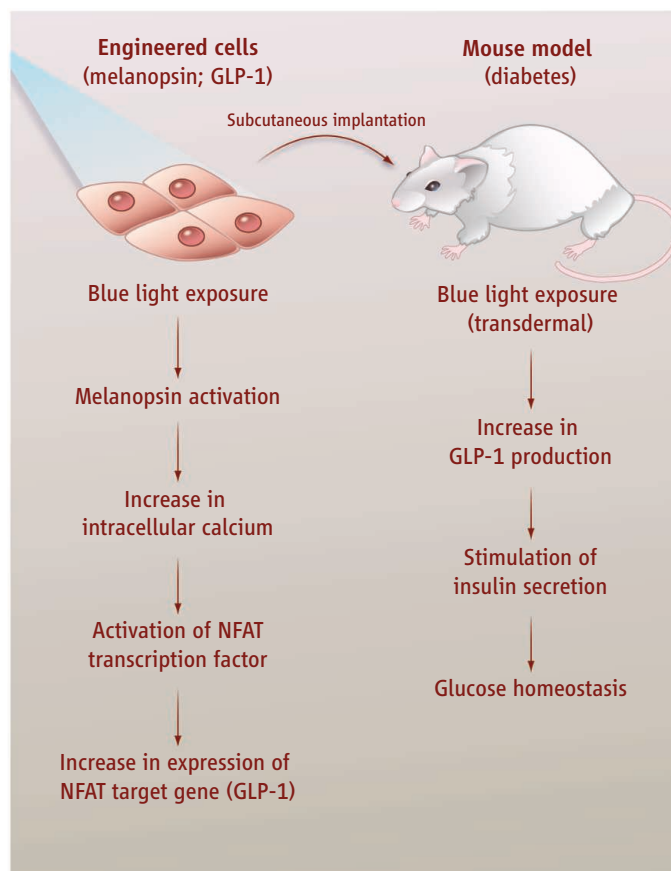
Optogenetic tools are DNA-encoded molecules that, when genetically targeted to cells, enable the control of specific physiological processes within those cells through exposure to light. These tools can pinpoint how these specific processes affect the emergent properties of a complex biological system, such as a mammalian organ or even an entire animal. They can also allow control of a biological system for therapeutic or bioengineering purposes. Many of the optical control tools explored to date are single-component reagents containing a photoactive signaling domain. An interesting question is raised by comparing optogenetics to synthetic biology. In the latter, interchangeable and modular DNA-encoded parts are assembled into complex biological circuits, thus enabling sophisticated logic and computation as well as the production of biologics and reagents (1, 2). Is it possible to devise strategies for the temporally precise cell-targeted optical control of complex engineered biological computational or chemical-synthetic pathways? Such a marriage of optogenetics and synthetic biology—which one might call synthetic physiology—would open up the ability to use optogenetics to trigger and regulate engineered synthetic biology systems, which in turn could execute computational and biological programs of great complexity (3). On page 1565 of this issue, Ye *et al.* (4) explore such a hybrid approach to controlling a biological system, as well as the bioengineering and preclinical capabilities opened up by such an approach.

Single-component optical control tools have been successfully devised, such as those based on naturally occurring light-gated ion

channels and pumps that organisms use to sense light. When genetically expressed in specific neurons within the mammalian brain, these tools enable the electrical activity of the targeted neurons to be driven or silenced by pulses of light (5, 6), thus revealing the causal roles that the targeted neurons play in behaviors and pathologies. As another example, a photoactivatable version of an intracellular signaling protein—the small guanosine triphosphatase (GTPase) Rac1—has recently been designed (7) in which a photosensor domain [the light oxygen voltage (LOV) domain] is fused to a mutated form of Rac1 such that the GTPase can be activated by light.

Ye *et al.* began with melanopsin, a heterotrimeric GTP-binding protein (G protein)—coupled light-sensitive molecule that, upon illumination, signals through the G protein subunit G_q to downstream effectors such as phospholipase C, protein kinase C, and calcium signaling. One of the outcomes of calcium signaling is the activation of gene

A marriage of optogenetics and synthetic biology could open the door to diverse applications, from animal models of disease to diagnostics and therapies.



Optogenetic control of mammalian physiology. Mammalian cells are engineered to express light-responsive melanopsin. Melanopsin activates a signaling cascade that activates the transcription factor NFAT, which drives the expression of a target transgene (GLP-1). When cells are implanted into mice and the animals are exposed to pulses of light, GLP-1 is produced. GLP-1 affects the production of insulin and can restore glucose homeostasis.

expression driven by a transcription factor called nuclear factor of activated T cells (NFAT). Ye *et al.* coexpressed the gene encoding melanopsin in cultured mammalian cells along with a DNA cassette encoding a reporter gene whose expression is controlled by NFAT (see the figure). Illuminating the cells for a few hours caused near-maximal expression of the reporter gene. The authors explored the application of this finding in a number of bioengineering and preclinical domains, including light-controlled bioreactors in which the cellular production of a genetically encoded payload placed under control of

the NFAT-dependent promoter can be driven by illumination. This allowed production of the payload to be regulated over time simply by modulating the patterns of light over periods of hours to days.

Ye *et al.* also show that this light-responsive melanopsin-NFAT signaling cascade can be induced in mice, placing, for example, the gene encoding glucagon-like peptide 1 (GLP-1) into the NFAT reporter cassette. Mammalian cells containing this cassette and the gene for melanopsin were generated and subcutaneously implanted into mice. Such mice, when exposed to blue light (transdermally), showed an increase in insulin production, as would be expected from the administration of GLP-1 (which stimulates glucose-dependent insulin secretion), and a concomitant decrease in blood glucose concentration as well. This demonstration shows that synthetic physiology tools can control organism states relevant to the understanding and treatment of conditions such as diabetes. Indeed,

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the authors performed this experiment in a mouse model of type 2 diabetes and show that beneficial changes in blood insulin and glucose concentrations arise when the diabetic mice, implanted with these engineered cells, are illuminated.

This study by Ye *et al.* shows that coupling an optogenetic driver to a downstream system can result in biologically meaningful changes. It also extends previous synthetic physiology results to use of such technologies in mammals *in vivo* with noninvasive stimulation (8). Relevant past work includes coupling a light sensor to transcription in bacteria (9), driving the heterodimerization of a DNA binding domain and a transcriptional activation domain by light to control gene expression (10, 11), and using the G protein-coupled light-sensitive molecule rhodopsin to actuate potassium currents in neurons in response to light, thereby enabling optical neural silencing (12). Ye *et al.* demonstrate that one of the most difficult aspects of designing and imple-

menting synthetic physiology is coupling the optogenetic tool to downstream signaling pathways. For example, using calcium to couple light reception by melanopsin to NFAT-driven transcription may result in unintended side effects of illumination, given the diverse cellular functions that calcium signaling controls. Clearly, synthetic physiology will need to devise more specific coupling strategies for connecting optogenetic tools to downstream synthetic biology processes.

The power of synthetic physiology approaches—leveraging the high speed and dynamic control of optogenetics and the computational power and biological richness of synthetic biology—may open up new applications, ranging from animal models of disease that are modulated by light, to personalized medicine applications in which cells from patients can be probed *in vitro* using causal tools to assess which pathways are involved in a given disease state. A tantalizing possibility also explored by Ye *et al.*

is whether these tools may be useful as components of a new generation of prosthetics, allowing ultraprecise correction of dysfunctional biological processes. Assessing optogenetic methods in nonhuman primates may be of use in exploring such translational possibilities (13, 14).

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PLANETARY SCIENCE

The Earth and the Sun

Robert N. Clayton

For more than 2 years, NASA's Genesis mission collected atoms of the solar wind (charged particles ejected from the Sun's atmosphere). Positioned at the Sun-Earth L1 Lagrange point about 1.5 million km from Earth, the spacecraft was well beyond the complicating effects of Earth's atmosphere and magnetic field, which hinder accurate ground-based astronomical measurements. The balance of gravitational forces at the Lagrange point allowed the spacecraft to maintain a fixed relationship to the Earth and the Sun with minimal expenditure of propellant. The highest priority of the mission was to determine the abundances of the stable isotopes of oxygen (^{16}O , ^{17}O , and ^{18}O) and nitrogen (^{14}N and ^{15}N) in the Sun and, by inference, in the whole solar system (1). However, on returning to Earth with its payload, the capsule suffered an unplanned hard landing in Utah in 2004, shattering most of the collector materials and thereby greatly complicating the initial sample analysis. After years of developing analytical techniques, McKeegan *et al.* and Marty *et al.*, on pages 1528 and 1533 of this issue (2, 3), reveal that these goals

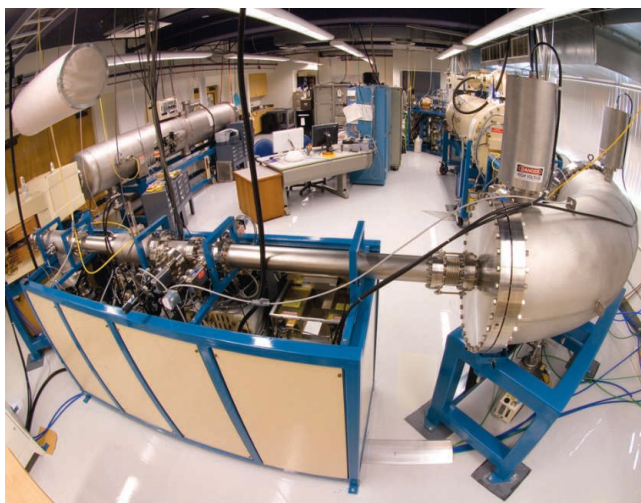
have now been accomplished.

Variations in stable isotope abundances have been studied in solar system samples (Earth, Moon, and meteorites), but interpreting this information has been thwarted by the lack of precise knowledge about the isotopic abundances of the initial material from which the elements evolved. It may be surprising that these initial isotopic abundances were not already known for such abundant elements (oxygen ranks third and nitrogen sixth in solar system abundance). Earlier

Sample collection by the Genesis spacecraft reveals the isotopic composition of elements in the solar system.

attempts were made to use lunar surface minerals as collectors of the solar wind (metal grains for oxygen, oxide grains for nitrogen), but the results were ambiguous and lacked adequate precision.

The solar wind is very dilute. The small number of atoms implanted in the collector material presents an analytical challenge, as illustrated by mass spectrometer count rates of the key rare isotopes ($^{17}\text{O}^{2+}$ and $^{12}\text{C}^{15}\text{N}^{-}$) of only 10 to 40 ions per second. In addition, the relatively low velocity of solar wind ions



From space to lab. The photograph shows the MegaSIMS facility at the University of California–Los Angeles used for oxygen isotope analysis of the Genesis samples (2, 3). The initial sputtering source for oxygen ions is in the far background; the cream-colored cylindrical structure at right is the tandem accelerator; in the right foreground is the electrostatic sector of the double-focusing mass spectrometer; in the left foreground is the magnetic sector; the large silver-colored cylindrical structure at the left contains the ion detectors.

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leads to penetration depths on the order of only 100 nm, so that cleaning of the sample surface must be done with great care. A further hurdle was presented by the large amount of solar wind hydrogen that was implanted in the collector material. For oxygen, this problem was solved by passing the ion beam, accelerated to energies of 1 MeV, through an argon “stripper,” to destroy hydride molecules, such as ^{16}OH (see the figure); for nitrogen, the problem was solved by the use of high-mass resolution to avoid interference by isobaric species.

The principal conclusion from the studies of McKeegan *et al.* and Marty *et al.* is that the Earth was not constructed from average solar system materials. Oxygen and nitrogen are major constituents of the Earth and must have been subjected to processes that alter their isotopic abundances. Although mass-dependent isotope fractionation of light elements, such as oxygen and nitrogen, is well known in terrestrial processes, such processes cannot be responsible for the observed variations: For oxygen, the ratios of $^{17}\text{O}/^{18}\text{O}$

are almost identical for the Earth and the Sun, whereas the abundance of the principal isotope, ^{16}O , is several percent smaller in the Earth than in the Sun; for nitrogen (with only two stable isotopes), the observed difference in $^{15}\text{N}/^{14}\text{N}$ ratio by a factor of almost 1.7 is far larger than any known mass-dependent isotope effect on Earth. It has been proposed that the large isotope effects in both elements are due to photolysis of their parent molecules (isoelectronic CO and N_2) in which the optical thickness of the abundant species ($^{12}\text{C}^{16}\text{O}$ and $^{14}\text{N}^{14}\text{N}$) greatly exceeds that of the rare species ($^{12}\text{C}^{17}\text{O}$, $^{12}\text{C}^{18}\text{O}$, and $^{14}\text{N}^{15}\text{N}$). This “self-shielding” process has been well studied in astronomical settings (4) and may have occurred in the early solar system.

Are there other elements that might exhibit similar behavior? Hydrogen, as H_2 , might also show self-shielding isotope effects, but this process cannot be observed by an Earth-Sun comparison, because deuterium (^2H) in the Sun has been destroyed by nuclear reactions (5). The element carbon is a special case: Although it is a chemical part-

ner with oxygen in the CO molecule, carbon has not shown any dramatically large isotopic variations. The absence of a large isotope effect in carbon can be understood in terms of the “isotopic scrambling” effect that occurs in the reaction $^{12}\text{C}^+ + ^{13}\text{C} \rightarrow ^{13}\text{C}^+ + ^{12}\text{C}$, because carbon, unlike nitrogen and oxygen, has an ionization energy less than that of the abundant hydrogen (6).

The results of McKeegan *et al.* and Marty *et al.* from the Genesis mission have demonstrated, once again, that return of solar system samples for study in terrestrial laboratories produces vastly better data than remote analysis methods.

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MOLECULAR BIOLOGY

A New Twist for Topoisomerase

Susana M. Cerritelli, Hyongi Chon, Robert J. Crouch

The worlds of RNA and DNA are not entirely separate. They frequently come together when RNA/DNA hybrids form during replication and transcription, which often induces genome instability (1–3). An enzyme called topoisomerase (Top) plays an important role in preventing RNA/DNA annealing during transcription (1). Another enzyme, ribonuclease H (RNase H), helps maintain genome integrity by removing RNA from hybrids (2, 3). On page 1561 of this issue, Kim *et al.* (4) report that Top1, one of the two types of topoisomerase, not only prevents the formation of often deleterious hybrid structures called R-loops, but also participates in the removal of single ribonucleotides (rNMPs), which are incorporated during DNA replication and have escaped the repair system. Using yeast, Kim *et al.* also show that, when there is a rNMP at the site of Top1 cleavage, the enzyme can create irreversible breaks in the DNA strand, assailing genome stability and perhaps contributing to disease.

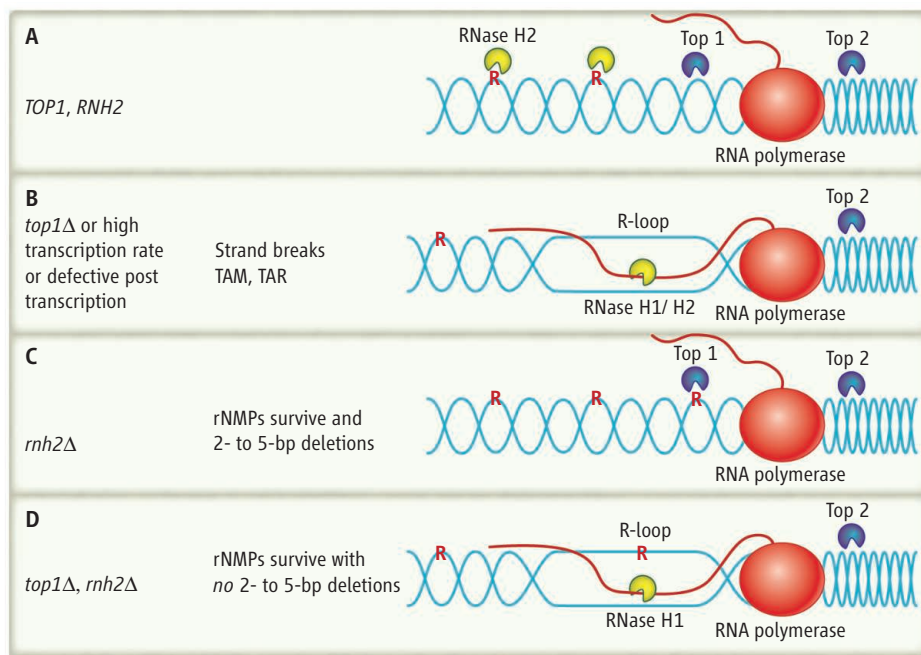
As RNA polymerases traverse DNA during transcription (producing RNA chains), distortions of the DNA can occur in front of and behind the polymerase (see the figure). The distortions cause torsion that compacts the DNA in front of the polymerase (creating a condition called positive supercoiling) and relaxes the DNA behind it (negative supercoiling) (5). Topoisomerases must perform molecular gymnastics to restore normal DNA topology (6). When Top1 is defective, nascent RNA can anneal to the underwound DNA and thus produce R-loops (see the figure). Similarly, R-loops can form when genes are transcribed at high rates (1), or if the proteins that normally interact with the nascent RNA are defective (2, 3). In most cases, the formation of R-loops has deleterious consequences, because the displaced DNA strand is left susceptible to breakage, which leads to transcription-associated mutation (TAM) (7, 8) or transcription-associated recombination (TAR) (2, 3). But when Top1 fails, RNases H can come to the rescue and remove R-loops by cleaving the RNA strand of the R-loop and restoring the original double-stranded DNA (1–3).

Topoisomerase 1 attacks on ribonucleotides in DNA leads to 2- to 5-base pair deletions.

There are two types of RNases H (H1 and H2), and both can process R-loops (see the figure) (9). *Escherichia coli* strains in which the genes encoding RNase H1 and Top1 are deleted are not viable. In *Saccharomyces cerevisiae*, *top1Δ* strains are viable when only one RNase H (either H1 or H2) is absent; in contrast, strains are unable to grow when all three enzymes (Top1, RNase H1, and RNase H2) are missing (10). RNases H and Top1 are not only linked through transcription and R-loop formation and removal; Kim *et al.* made the intriguing observation that the enzymes are also involved in processing rNMPs embedded in DNA, which are created by a recently described misincorporation error during replication (11).

The inclusion of rNMPs into DNA during replication is influenced by a number of factors. For example, DNA polymerase incorporation of ribonucleoside triphosphates (rNTPs) is affected by the high ratio of rNTPs to deoxynucleoside triphosphates (dNTPs) in vivo, the inherent preference of each DNA polymerase for dNTPs, and the system's ability to remove rNMPs once incorporated into DNA (12). In vitro experi-

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On top. (A) In yeast wild-type cells (TOP1, RNase H2), R-loop formation is prevented when Top2 relieves the positive supercoiling ahead of the transcribing RNA polymerase, and Top1 relieves the negative supercoiling behind. RNase H2 cleaves rNMPs incorporated into DNA, which leads to their replacement with dNMP. (B) In *top1Δ* mutants, RNA/DNA hybrids are formed and processed by RNase H1/H2, and rNMP removal is initiated by RNase H2. Mutations can result by TAM and TAR. (C) In *rnh2Δ* cells, Top1 will encounter and cleave rNMPs that have not been removed because RNase H2 is missing, which produces 2- to 5-bp deletions. (D) In the double mutant *top1Δ rnh2Δ*, RNA in R-loops may be cleaved by RNase H1, but rNMPs will remain in the DNA. DNA is blue, RNA is red, and single rNMPs in DNA are noted as red R.

ments suggest that more than 10,000 rNMPs are incorporated during the replication of the ~12.5 million-base pair (bp) *S. cerevisiae* genome. The lack of abundant rNMPs in normal DNA, however, implies that they are quickly and efficiently removed. RNase H2 seems to initiate the removal process (11), which most likely continues on a pathway involving Fen1 nuclease, a structure-specific nuclease that plays an important role in maintaining genome stability (13). In contrast, in the absence of RNase H2, rNMPs persist in abundance in genomic DNA, and are associated with the accumulation of 2- to 5-bp deletions within tandem repeats; these deletions may be due to removal of rNMPs by an alternative pathway that does not require the post-replicative repair system that fixes sequence mismatches, which suggests repairs outside of DNA replication (14).

Researchers have previously reported this rare type of mutation in association with both Top1 activity and high rates of transcription (7, 8). These reports prompted Kim *et al.* to ask whether Top1 could be involved in generating the 2- to 5-bp deletions seen in RNase H2-defective strains. They found that eliminating Top1 activity prevents the deletions that occur when RNase H2 is absent, which suggests that deletions are a signature of

Top1 cleavage of rNMPs. Such deletions in the *CAN1* gene (15) were observed by TAM and in RNase H2-defective strains, but concordance of deletions was not perfect. Only two of the three TAM deletion hotspots in the *CAN1* gene were observed in *rnh201Δ* strains (one of the subunit-encoding genes of RNase H2).

Top1 may encounter ribonucleotides in DNA because of high transcription rates that prevent the access of RNase H2, or when RNase H2 is defective (see the figure). Kim *et al.* convincingly showed that if there is a ribonucleotide at the site of Top1 cleavage, an irreversible single-stranded DNA (ssDNA) break occurs. Top1 of *S. cerevisiae* nicks and seals only one of the DNA strands, with the active site tyrosine forming a phosphodiester bond with the 3'-phosphate of the DNA end. After hydrolysis, the phosphodiester bond between two deoxynucleotides dNMPs is restored, which reforms the natural 3'-5' connection. However, when Top1 cleaves between a ribo- and deoxynucleotide, the 2'-hydroxyl (OH) of the ribose permits formation of a 2'-3'-cyclic phosphate rather than the usual 3'-5' phosphodiester, prohibiting religation and producing a stable ssDNA break. Deletions of 2- to 5-bp occur in tandem repeat

sequences in the DNA, which suggests that repairing the ssDNA break within a tandem repeat can induce realignment and removal of some of the repeat sequence. However, most of the rNMPs in DNA persist, which indicates that removal of all rNMPs is not a normal function of Top1 (see the figure), and may be detrimental.

The results suggest that short tandem nucleotide repeats and rNMPs in DNA are necessary, but not sufficient, for deletions. Not all such repeats are deletion hotspots, and some hotspots are different in strains with TAMs and defective RNase H2. What accounts for these findings? Are the rNMPs misincorporated by DNA polymerases the sole targets for Top1 and, if so, is the race between Top1 and RNase H2 affected by transcription rates? In addition, not all rNMPs in DNA are Top1 targets. How are the Top1-resistant rNMPs in RNase H2-deficient strains copied and/or removed?

Mutations in human RNase H2 can cause a rare autoimmune disorder, Aicardi-Goutières syndrome (AGS), which involves severe neurological defects and often leads to early childhood death (16). Kim *et al.* suggest that AGS patients with altered RNase H2 may suffer from effects of cumulative deletions similar to those seen in *S. cerevisiae rnh201Δ* strains. It will be of considerable interest to see if 2- to 5-bp deletions are more frequent in AGS patients.

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NEUROSCIENCE

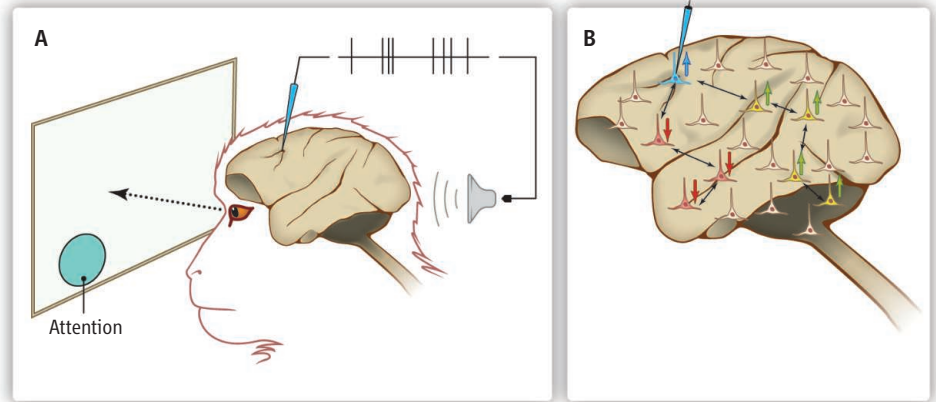
Attention—Voluntary Control of Brain Cells

Pieter R. Roelfsema^{1,2}

Monkeys are able to increase and decrease the activity of single neurons involved in vision.

Suppose that you were given feedback about the activity of one of your brain cells, so that you could listen to its activity and monitor the electrical impulses it uses to communicate with other neurons. Would you be able to control its activity by changing your thoughts? How would you do it? These are the questions that Schafer and Moore (1) begin to answer on page 1568 of this issue. They show that trained monkeys can increase or decrease the activity of single neurons in the frontal eye field, an area of the cortex involved in generating eye movements (2) and controlling spatial attention. If a monkey shifts attention to a particular location in space, the frontal eye field neurons linked to that location increase their firing rate (3). The study demonstrates a promising technique for elucidating the role of other neurons during cognitive tasks and suggests that “mind control” might be useful in treating brain disorders.

In Schafer and Moore’s experiment, monkeys looked at a computer screen while hearing a tone that informed them about the activity of one cell in their frontal eye fields. In one phase of the experiment, they were rewarded if they could increase the activity of the cell; in another phase, they were rewarded if they could decrease activity. The animals were able to control the neuron’s activity, although they typically changed it by only a small fraction (6% on average). How did the monkeys achieve this control? During the task, the monkeys had to look steadily at a fixed point on the screen; the researchers found no measurable effect of mind control on eye movements. However, they did find an effect on visual attention (the selective concentration on one spatial location). When the cell’s activity increased, the animal was better able to detect a visual object at the location coded by the cell, as if the monkey directed his attention to this location if he wanted to increase the neuron’s activity. This finding



Mind control. Experiments using monkeys (1) show that animals that receive auditory feedback about the activity of a single neuron involved in vision can increase or decrease the cell’s activity. Such results suggest that mind control could be used to step-up the deployment of spatial attention (A). Mind control of a single neuron (B) shown in blue is likely to increase (green) or decrease (red) the activity of neurons in other brain regions. Mapping these networks could provide new insights into the functions of attention.

supports the view that this brain area has a role in determining the focus of attention.

Mind control of single cells is not unique to the frontal eye fields; research has shown that monkeys and humans can also exert control over neuronal activity in other brain regions. In a classic experiment, Fetz and Finocchio (4) trained monkeys to control the activity of neurons in the primary motor cortex. The monkeys initially made muscle contractions to increase the activity of motor neurons. Later, these animals learned to withhold the contractions while still being able to control the cells’ activity. We do not know how these monkeys learned to control their brain activity, but it is possible that they were thinking about a particular movement without actually carrying it out. This type of control over neuronal activity in the motor cortex and in the parietal cortex (5, 6) can be used to steer prosthetic devices, and people with paralysis could be aided by future advances in this field (7). Already, monkeys can learn to control a robotic arm, and feed themselves, by varying the activity of a larger population of cells in motor cortex (8).

Mind control also occurs outside the neural systems that control movement. A recent study (9) investigated mind control of single neurons in people who had electrodes implanted in their brains as part of a procedure to treat epilepsy. The study focused on

the medial temporal lobe, which is involved in generating memories of facts and events. Some neurons in this region code the identity of famous people and individuals that the subject knows (10). One of the cells in this experiment responded well to a picture of Marilyn Monroe. When the subject saw a blend of two pictures, one of Marilyn Monroe and the other one of an actor that did not activate the cell, he could increase the cell’s activity by focusing on the concept “Marilyn Monroe.” In contrast, he could suppress the cell’s activity by focusing on the other actor. This mind control of neuronal activity in the medial temporal lobe worked well, as subjects were able to change the activity of cells by a factor of 6, on average.

These studies, taken together, demonstrate that we can indeed control the activity of cells in many parts of our brain. An aspect that links these studies is the role of attention. Subjects controlled the activity of cells in the medial temporal lobe by focusing attention on one famous individual, and they controlled the activity of cells in the frontal eye fields by focusing attention onto a specific region of visual space (1). In both cases, cortical cells become more active when subjects focused attention on the relevant concept. These effects of attention on neuronal activity are widespread in the cerebral cortex. They occur in primary sensory areas (11) and

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in areas of the temporal lobe (9), parietal lobe (12), and frontal lobe (3), as well as at subcortical processing levels (13). If a concept is in our mind, even momentarily, neurons in all areas that code properties of this concept enhance their activity at that precise point in time. As a result, the evolution of attentional modulation over time can provide direct insight into the sequence of mental processing steps needed to solve complex cognitive tasks (14). Every cognitive task requires the joint activation of many brain areas that need to exchange information, and the widespread nature of attentional signals suggests that they could provide the vehicle for this information exchange (11).

Controlling the activity of a particular cell must be associated with changes in the activity of other brain regions that are either necessary for exerting the mind control or that change activity as a consequence. To better understand the functioning of these large-scale attentional networks (see the

figure), future studies could combine mind control of cells in one area with monitoring activity in another.

It is also exciting to anticipate the potential applications of mind control for patients. Foremost are the potential prosthetic applications for people with paralysis. But researchers are also exploring the possibility of using mind control to treat other disorders. Subjects can control patterns of brain activity that can be measured noninvasively with magnetic resonance imaging or electroencephalography (15). Could depressed patients improve their mood if they are trained to increase the activity of their brain pleasure networks? Neuroscientists are entering into an era where their discoveries are not only of importance to understand brain and mind, but where their findings will be increasingly used to treat brain disease.

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PHYSICS

The Limits of Ordinary Matter

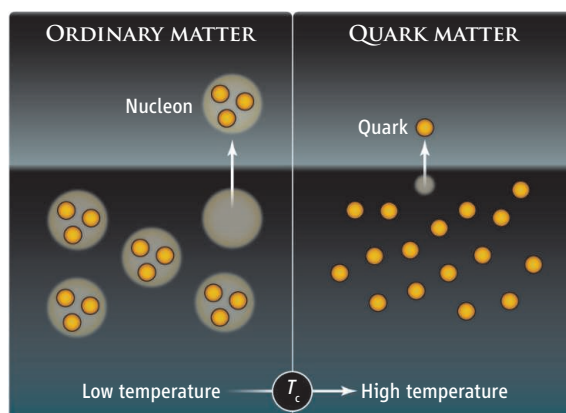
Berndt Müller

All ordinary matter consists of protons and neutrons, collectively called nucleons, which are bound together in atomic nuclei, and electrons. The elementary constituents of protons and neutrons, the quarks, almost always remain confined inside nucleons (or any other particle made up of quarks, called hadrons). The fundamental force that binds quarks together—the strong, or “color” force—cannot be overcome unless extremely high-energy conditions are created, such as through heavy-particle collisions. Theoretical simulations based on quantum chromodynamics (QCD) predict that the transition temperature for the appearance of free quarks should occur at 2.0×10^{12} K (an energy of 175 million eV) (1, 2). Since 2000, the Relativistic Heavy Ion Collider (RHIC) at Brookhaven National Laboratory has created the necessary conditions to form quark mat-

ter in particle collision, but determining the transition temperature under these conditions is challenging. On page 1525 of this issue, Gupta *et al.* (3) show that the relevant temperature and energy scales can be extracted from recent experimental studies and find that the transition temperature is in remarkable agreement with theory.

Detailed information about the nature of

The temperature at which quarks escape the confines of protons and neutrons has been determined from high-energy particle collision data.



Matter rearrangements. In ordinary matter, quarks can be added or removed only in triplets, but in quark matter, they can be added or removed individually. Gupta *et al.* show how properties associated with such changes can be used to determine the temperature of the transition between these two regimes from particle collision data.

the transition can be obtained from theoretical studies of the change in free energy when a quark is added or removed from a finite piece of matter. The free energy function is needed because it includes the effects of temperature and entropy, as well as particle number. In the case of ordinary matter, quarks can be added or removed only in the form of nucleons, that is, in triplets; after the transition to quark matter, quarks can be added or removed individually (see the figure).

This difference in how particles are added manifests itself in an abrupt change in the response of the matter to changes in conserved quantum numbers carried by quarks, in particular, the baryon number (a measure of relative number of quarks and antiquarks). In statistical physics, the response of a system to infinitesimal changes is measured by coefficients called susceptibilities, which carry the information about the nature of the constituents of the matter. The susceptibilities for baryon number and similar conserved quantum numbers, such as electric charge, are predicted to change drastically and characteristically when ordinary matter transitions to quark matter, providing a distinctive fingerprint of the transition (4, 5). For example, a certain ratio of baryon number susceptibilities (the kurtosis, a

measure of the shape of the probability distribution) drops to one-ninth of its normal value over a narrow temperature range.

Not only are enormous accelerators needed to give colliding particles the required energy to create quark matter—a sufficiently large chunk of this extremely hot matter also must be maintained by inertial confinement for a sufficiently long period of time to ensure thermalization and permit the observation of its properties. Experiments conducted at RHIC achieved the needed energy by colliding two large nuclei, such as those of gold, against each other at energies up to 2 trillion eV (2 TeV) (6). These studies have shown, surprisingly, that quark matter is not a dilute gaseous plasma of quarks and gluons, the particles that carry the strong force. Instead, a strongly coupled liquid with almost no viscosity, that is, an almost ideal fluid, forms. The emission pattern of protons provides indirect evidence that quarks move independently inside this hot matter.

Direct evidence for the transition temperature and the nature of the transition can be extracted from the data by examining various susceptibilities. Susceptibilities are related to fluctuations of the thermodynamic variables that occur within a fixed volume of matter. In the case at hand, the baryon number susceptibilities can be related to the fluctuations of the number of protons emitted even by event in nuclear collisions (4). The emitting volume is not known precisely, so it is necessary to study ratios of baryon susceptibilities corresponding to different orders in the expansion of the free energy (7, 8). These ratios change during the transition from ordinary to quark matter in a characteristic manner and can be used to track the change in the structure of the matter with the change in thermal conditions as the nuclear collision energy is varied (9).

Gupta *et al.* went one step further. Numerical QCD simulations of interactions among quarks are carried out for a discretized version of space-time, an approach known as lattice gauge theory. To determine the transition temperature, the lattice simulations must fix the energy scale, which is usually done by comparison with some other observable quantity determined directly or indirectly from experiment. The authors show that the scale, and thus the actual value of the QCD transition temperature, can be determined from the same data used to compare with predictions for the quark susceptibilities, if the data are known over a sufficiently wide parameter range. This new method has been made possible by recent results from the STAR collaboration at RHIC (10), which probed matter with the ratio of the baryon

chemical potential (the change in energy of the system as the net number of quarks minus antiquarks fluctuates) to the temperature extending over a wide range by varying the collision energy. The values of the temperature and chemical potential reached at each energy were obtained by comparing the measured particle yields with those calculated in the hadron resonance gas model. The value for the transition temperature obtained by Gupta *et al.* (175 $\pm$ 1 MeV) agrees very well with the values obtained by comparison with single-hadron properties.

With many more results expected from the RHIC experiments in the near future, additional insights into the details of the transition from ordinary matter to quark matter are within reach. To fully make use of the data and confirm the conclusions presented by Gupta *et al.*, it will be necessary to develop a

comprehensive theoretical framework for the creation, evolution, and freeze-out of thermodynamic fluctuations in the matter produced in nuclear collisions that moves at relativistic speeds. This challenge to theorists is commensurate to the experimental challenges that have been overcome by recent detector and accelerator upgrades at RHIC.

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ECOLOGY

Why Did You Lévy?

Daniel Grünbaum

Mussels in intertidal beds use random movement behaviors to optimize formation of patches to balance feeding and mortality.

Nature is full of biological landscapes that seem static to the casual observer, but actually contain highly dynamic spatial features. These features are shaped dramatically, if slowly, by subtle interplays between large-scale population-level patterns and small-scale movements of individual organisms. For spatial ecologists, a challenging aspect of such landscapes is the chicken-and-egg character of the underlying interactions: Organism movements are dictated by the environment, but the environment is strongly affected by organism movements. On page 1551 of this issue, de Jager *et al.* (1) reveal how, for mussels in patchy intertidal beds, the ecology of dynamic spatial patterns and the evolution of movement strategies are tightly linked.

Mussels attach to substrate and to each other using byssal threads, a biological material with remarkable mechanical properties secreted by the mussel's foot (2) (see the figure). By systematically adding and removing threads, mussels adjust their position and, especially as juveniles, are surprisingly mobile. For those of us to whom "perfection

in movement" more readily connotes sprinting cheetahs and soaring albatrosses, de Jager *et al.* have a surprising message: Despite their leisurely locomotion—or perhaps because of it—mussels exhibit economy of movement and savvy behavioral strategies that approach a theoretical ideal as well as, or better than, more visibly athletic species.

De Jager *et al.* combined experiments and theory to quantify relationships between mussel movement behaviors and the patchy distributions in which mussels are often found. Individual mussels have reduced mortality when they are in the immediate vicinity of other mussels, probably because they are less likely to be torn away by currents (3, 4). However, mussels filter-feed on algal cells and other small plankton, and large masses of mussels deplete local food resources. Hence, mussels with many immediate neighbors but few more-distant neighbors—that is, those that are aggregated on short length scales but dispersed on longer length scales—enjoy the best of both worlds. Patchy distributions provide individuals with just this combination of neighbors.

If most members of a population benefit from patchy distributions, it seems inevitable that evolution would shape mussel behavior to produce patches. However, population

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Footloose and scale free. (Left) Mussels form patchy distributions that favorably balance mortality risk and food availability within intertidal beds. (Lower right) Mussels move by attaching and detaching byssal threads laid down by the foot. (Upper right) The resulting patches are due to random movement behaviors called Lévy walks that optimize formation of intermediate-sized groups.

distribution is an emergent phenomenon—a collective feature resulting from the decentralized behaviors of many individuals. An individual mussel cannot directly create a favorable patch structure—it can only join or abandon patches it encounters in its meanderings, according to its own evolutionary interests. Favorable patch structure can emerge only if behavior that promotes favorable patches benefits individuals more than alternative behaviors promoting other spatial distributions.

This is a high barrier: Nature (and human experience) is full of situations in which average payoffs could be increased by group-oriented behaviors, but those behaviors either don't occur or occur only in context-dependent or compromised forms because individuals benefit more immediately by being selfish. De Jager *et al.* demonstrate that in mussels this barrier is overcome: Observed mussel behaviors create favorably patchy mussel distributions and, according to a fitness metric evaluated by de Jager *et al.*, these behaviors are indeed more beneficial than all potential behavioral variants.

Like most organisms, mussels occupy habitats that vary at large scales, but they sense their environments only on much smaller scales. To cope with this mismatch, juvenile mussels searching for suitable substrate use “random walks”—behaviors in which elements of movement (speed, direction, persistence time, etc.) are selected at intervals from statistical distributions of random values (5). Random walks are common among nearly all motile organisms, and are ecologically important because they link environmental heterogeneity, biomechanical constraints on locomotion and sensing, and

clever behavioral strategies enabling organisms to circumvent these constraints while exploiting variable environments.

In the simplest types of random walks—for example, the Brownian motion of small inanimate particles—statistical distributions of movements are exponential. De Jager *et al.* show that mussels are among the growing number of organisms known to employ a more complex type of random movement—a Lévy walk—in which movements have power-law distributions (6). Behaviorally, this is intriguing because unlike exponential distributions, power-law distributions require physiological or cognitive memory of the duration of the current move. Hence, it seems likely that organisms that perform Lévy walks must have specific adaptations for doing so. Ecologically, Lévy walks are important because they yield a “scale free” blend of long and short movements that can be more efficient than exponential distributions in locating and exploiting resources that are patchy at multiple scales. Notably, in mussels this efficiency extends beyond exploitation to the fundamental mechanisms underlying formation of favorably patchy mussel beds.

De Jager *et al.*'s suggestion that mussel behavior balances effects of short- and long-range aggregations implies specific predictions about how present and future environmental variations affect mussel distributions over ecological and evolutionary time scales. More intense feeding competition or lower plankton abundance yields smaller or more widely spaced patches. Conversely, higher-flow environments enable larger patches. If observed behavioral responses to food limitation indicate a broader strategy for miti-

gating environmental stresses, de Jager *et al.*'s behavioral analysis suggests that future oceans may induce compensatory shifts in mussel beds. For example, higher temperatures, which typically lead to decreased dissolved oxygen and increased respiration, may increase respiratory stress inside mussel aggregations. Low pH may make formation of calcium carbonate shell more difficult inside large mussel patches. If so, climate change and ocean acidification may render today's mussel patch structure unviable.

Insights into the present and future of biological patchiness are strongly needed. Theoretical ecology, climate modeling, and resource management rely heavily on “mean-field” models, which intentionally average out small-scale patchiness (7). These models use mathematical relationships—functional responses—describing how the average density of a population changes over time as a function of averaged prey, predator, and competitor populations. If we knew the correct functional responses, we could predict how populations fluctuate in nature and, consequently, understand the basis of fundamental ecological phenomena such as productivity, stability, coexistence, and diversity.

Patchiness causes major problems for mean-field models, because populations with the same average density but different patch structure can have very different ecological dynamics. In mussels, and perhaps many other species, it appears that we can anticipate patch structure by quantifying behavior. Studies like de Jager *et al.*'s that unify individual behavior with landscape-level distributions are key to future ecological models that better reflect how patchy populations differ from uniform ones and more accurately predict these populations' responses to environmental changes.

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SPORE* SERIES WINNER

Computational Experiments for Science Education

Charles Xie,[†] Robert Tinker, Barbara Tinker, Amy Pallant, Daniel Damelin, Boris Berenfeld

Computational physics, which provides digital representations of natural phenomena by solving their governing equations numerically, has transformed areas as diverse as scientific research, engineering design (1), and film production (2). It is also changing the way science is taught. The *Molecular Workbench* (MW) software, <http://mw.concord.org>, developed by the Concord Consortium, illustrates this perspective. MW models atomic-scale phenomena on the basis of molecular dynamics and quantum mechanics calculations, which enables students to carry out computational experiments to investigate and learn a wide range of science concepts.

The atomic world is alien to students: Electrons, atoms, and molecules are too small to be seen, and their interactions resemble nothing in the everyday observations that shape our intuitions. In the world of electromagnetic forces, thermodynamics, and quantum mechanics, there is little that students can assemble or tear apart with their bare hands in order to learn how those rules work. Traditional static ball-and-stick models of molecules fall short of conveying those essentially dynamic rules.

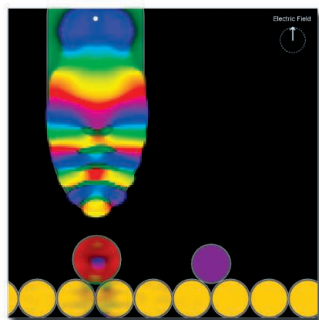
When direct hands-on experiences are not feasible in the classroom, computational experiments provide attractive alternatives. Inquiry through computational experiments is similar to inquiry through real experiments: Students observe visualizations, raise “what-if” questions, formulate hypotheses, design and conduct investigations to test their ideas, and, finally, analyze and interpret results. In some cases, good simulations can be just as effective as their real counterparts (3, 4).

For a computer simulation to become a

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Scanning tunneling microscopy simulation. Using MW’s quantum dynamics engine, the tunneling current from the probe tip to an atom sitting at the surface of a substrate is dynamically visualized. The electron wave is colored by the phase.

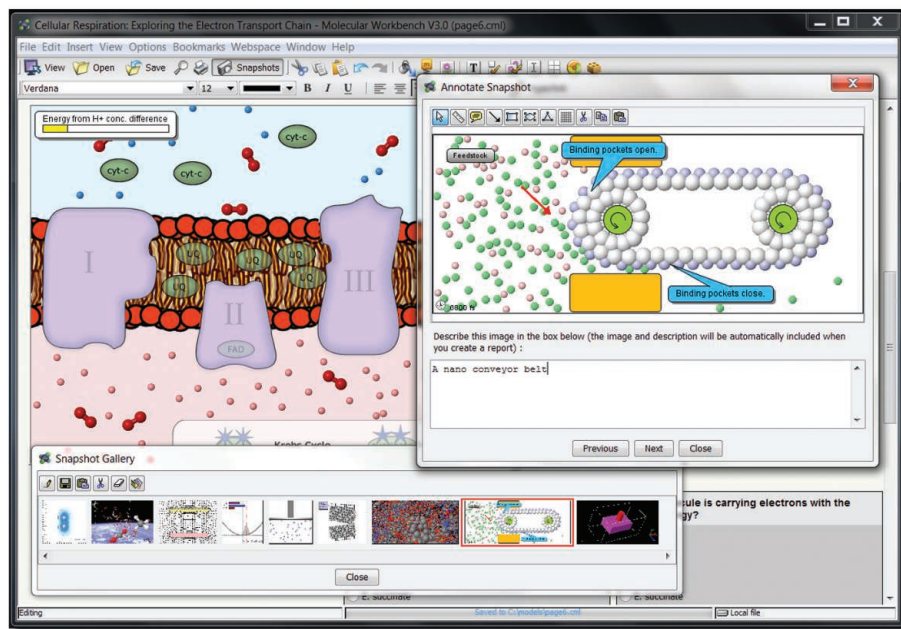
versatile experimentation tool, a computational engine capable of accurately simulating many real-world situations is needed. Each instance of such a generic engine models a specific phenomenon. For example, the computer models of a pendulum and a spring are two engines for solving Newton’s equation of motion. The two appear to be different, but they are computed using the exact same engine.

MW is a tool for designing and conducting computational experiments with atoms and molecules, based on molecular dynamics and quantum dynamics simulation methods that originate from molecular modeling research (5). These computational methods approximate the fundamental laws in the world of atoms and molecules, and so MW’s computational engines

Computational experiments based on solving fundamental physics equations bring authentic science to the classroom.

create dynamic visualizations of atomic motions and electron waves (see the first figure). The molecular dynamics engine uses classical mechanics to predict the motion of atoms according to the forces computed from potential energy functions that model interatomic interactions (6). The quantum dynamics engine solves the time-dependent Schrödinger equation to predict the propagation of wave functions in potential fields that model atomic-scale structures (7). The engines can be configured to simulate real or thought experiments. Users can intervene in the calculation by changing variables as the engines run. The results are visualized instantaneously, which allow users to observe and interact with the emergent phenomena. Using MW’s capacity for embedding computational experiments in curriculum materials, we and other MW users have created hundreds of classroom-ready interactive online lessons that have been widely used.

The computational engines in MW cover a broad scope of science topics, including gas laws, states of matter, chemical bonding, chemical reactions, electrostatics, heat transfer, fluid mechanics, fractures, quantum



Simulation snapshot. An image taken from the simulation of a hypothetical nano conveyor belt designed to selectively transport molecules across a membrane.

mechanics, diffusion, osmosis, self-assembly, and so on (see the second figure). The fact that many seemingly disparate phenomena emerge from a couple of computational engines that employ only a few general scientific laws demonstrates the unity of science, a profundity noted by physicist Richard Feynman in his famous lecture that opened the field of nanotechnology: "I am inspired by the biological phenomena in which chemical forces are used in repetitious fashion to produce all kinds of weird effects" (8). This unity is key to addressing the problem in U.S. science curriculum, famously described as "a mile wide and an inch deep" (9). Recognizing this problem, the Conceptual Framework for the New Science Education Standards has put forward a "more coherent vision" to move in the direction of "fewer, higher, clearer" standards (10).

An integrative tool such as MW can support novel instructional strategies that focus on the conceptual unity of otherwise fragmented factual knowledge. For example, the microscopic pictures and the macroscopic properties of the three states of matter are typically taught as individual facts. But all of them can be explained and connected using a single computational experiment with a particulate model: Students add particles into a container under a piston, adjust their properties, and run molecular dynamics simulations to see what happens. They discover that gas particles move freely to assume the entire volume of the container, liquid particles flow to occupy the lowest part of the container, and solid particles vibrate and maintain a fixed volume and shape. When pushing the piston, they find that a gas can be significantly compressed, whereas a liquid or a solid can hardly be. Gradually raising the temperature of a solid, they observe how it softens, collapses, melts, evaporates, and expands. They can even fiddle with the interaction potential among particles to study how it is responsible for the formation of each phase. Such a computational experiment unites many different ideas and provides the micro-macro connections needed for developing a deeper, more coherent understanding.

The fundamental physical laws used to build MW's engines ensure the accuracy and depth of many computational experiments they support. For example, the First and Second Laws of thermodynamics, the Boltzmann distribution, Fourier's law of heat conduction, Raoult's law, Pascal's principle, Archimedes's principle of buoyancy, and the ideal gas law can be discovered or tested. This capacity extends learning to the level of quantitative analysis, which is an

important part of scientific experiments.

An open-ended tool such as MW allows students to construct their own artifacts to generate and evaluate their own ideas. Learning through creating a computational experiment to answer a challenge complements learning through interacting with an existing one (11). This approach sets a higher goal for students and often motivates them to learn more actively. For example, when teaching the ideal gas law ($PV = Nk_B T$), instructors can ask students to design computational experiments to investigate a number of interesting questions, such as, "Why doesn't the law include molecular mass as a factor?" and "Why should N be the number of molecules but not the number of atoms?" These questions are hard to answer without a mastery of complex mathematics and statistical mechanics. But with MW's graphical user interface, students can bypass those barriers and come up with computational evidence and explanations. What would have been difficult to accomplish is simplified to visual manipulations.

General and physical chemistry students in a pilot study produced nearly 150 computational experiments to explore principles and applications in chemistry; such as ionic bonding, purification, and fuel cells. When challenged to come up with designs that break the ideal gas law, students created surprising solutions. They studied factors ranging from molecular size and mass, the strength and range of the van der Waals attractions, to covalent bonding. One student even meticulously constructed a setup that showed the correlation between the gas laws and the law of energy equipartition. The creativity of these students was unleashed. Their experiences of learning chemistry were transformed into fruitful science explorations.

MW represents a successful application of computational physics to developing effective learning tools. The generations of computational scientists who contributed to the theory and practice of the subject presumably did not anticipate that one day their techniques would benefit thousands of schools all over the world. In fact, education and research

About the Authors



Charles Xie is the computational physicist who created the Molecular Workbench. Robert Tinker is the founder of the Concord Consortium. Barbara Tinker was the project manager. Amy Pallant is a senior educational researcher. Daniel Damelin is a senior curriculum developer. Boris Berenfeld was a senior scientist. Robert and Boris served as the principal investigator (PI) and co-principal investigator of several molecular literacy projects that developed the molecular dynamics engine. Charles served as the PI of a nanotechnology project that developed the quantum dynamics engine." **Group portrait:** A. Pallant, D. Damelin, B. Tinker, B. Berenfeld, C. Xie, and R. Tinker.

share a common goal: to understand how the world works. It is, therefore, not a surprise that a research technique can be successfully converted into a learning tool. This makes us wonder how many other tools invented by scientists and engineers are still waiting to be "discovered" by educators and translated into educational technology.

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MEDICAL RESEARCH

To Improve Health Care, Experts Look to Cure Clinical Trials

Clinical trials are the systematic way that modern medicine determines whether new treatments are safe and effective. But while the process is critically important to improved health care, the number of new drugs submitted to the U.S. Food and Drug Administration in recent years is only about half what it was in the 1990s.

To experts at a conference cosponsored by AAAS, this is symptomatic of a system ill suited for 21st-century medicine. Today's clinical trials, in an effort to measure the often subtle effect of new therapies, tend to be huge and unwieldy. Now the search is under way for more streamlined, cost-effective, and sensitive ways to assemble patients and collect data.

study will be conducted, the type of patients recruited, and trial results.

The Web site makes this information public in a structured format for the very first time, said its director, Deborah A. Zarin, "thereby ensuring objective and comprehensive reporting and mitigating opportunities for positive 'spin' of study results."

PatientsLikeMe, a privately owned Web site, aims to empower patients in the clinical trial process by making it easier for them to share information, said Paul Wicks, the site's research and development director.

He described how a group of patients with amyotrophic lateral sclerosis greeted initial reports that low doses of the drug lithium carbonate slowed progression of their disease. One patient located a paper abstract in Italian, used Google software to translate it into English, and shared it online.

"Before it was even published [in English], there were more patients in our system taking lithium prescribed for them by their doctors than were in the [Italian] clinical trial," Wicks said. While the treatment proved ineffective,

the response showed how patients might actively engage in managing their own care.

Participating in a clinical trial means multiple visits to researchers, and many patients decide that it is not worth the hassle. But the pharmaceutical company Pfizer used the conference to introduce its test of the first virtual clinical trial, using a drug approved for bladder control.

Web-based and mobile phone apps are being used to manage recruitment, enrollment, data entry, and monitoring. Blood draws can be scheduled online, and the drug is shipped directly to the patient. Physician assistance is available 24/7 by phone.

Some conference participants expressed curiosity but also unease with a virtual approach that could eliminate face-to-face contact with study patients.

Another strategy for reducing the size and complexity of future trials could be to examine how a new drug or device works at the cellular or molecular level, Califf said, and evaluate these responses in patients. This approach could help researchers better anticipate risks and benefits earlier in a trial.

"It is critical that we consider how these developments will affect the more traditional ways that clinical research has been done," said Mark S. Frankel, director of AAAS's Scientific Responsibility, Human Rights and Law Program, "and what that will mean for translating findings into treatments."

—Bob Roehr

AAAS

New Dues for 2012

The AAAS Board of Directors has approved a dues increase for 2012. The Board authorizes increases to cover two kinds of expenses: unavoidable costs associated with running AAAS and publishing *Science*, and new expenses that add value to membership. Postage and paper increases and improving online resources are examples of the kinds of expenses the Board anticipated in setting the 2012 rates.

The new rates are effective for membership terms beginning after 31 December 2011. As listed below, they do not include postage or taxes for international members, which is additional.

Regular professional members	\$149
Postdocs and K-12 teachers	\$99
Emeritus members who receive print <i>Science</i>	\$115
Students	\$75
Patrons	\$310
Institutional rate for print for high schools and public libraries	\$360
All other institutions receiving print	\$1079

For further information, including subscription rates for *Science* online, librarians should contact AAAS or their subscription agents, or visit the *Science* Web site at www.sciencemag.org.

All members will be advised of the new dues rates on their renewal notices for 2012. Member dues and voluntary contributions form the critical financial base for a wide range of AAAS activities. For more information, contact the AAAS Membership Office at 202-326-6417 or visit www.aaas.org/membership/.



"Our clinical trials system is falling behind the needs of society, and we have to figure out a way to do it more efficiently and better," said Duke University's Robert M. Califf, a leading U.S. expert on the process.

He delivered this blunt assessment in a keynote address to more than 200 participants at "Clinical Trials: Present Challenges and Future Opportunities," a conference held 6 to 7 June on the campus of the National Institutes of Health (NIH). The National Library of Medicine and the Friends of the National Library of Medicine joined AAAS as cosponsors.

When the National Library of Medicine created www.clinicaltrials.gov in 2000, the Web site was seen as revolutionary. It now includes most new U.S. clinical trials and many overseas, detailing where and how a

Human Fatty Liver Disease: Old Questions and New Insights

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Nonalcoholic fatty liver disease (NAFLD) is a burgeoning health problem that affects one-third of adults and an increasing number of children in developed countries. The disease begins with the aberrant accumulation of triglyceride in the liver, which in some individuals elicits an inflammatory response that can progress to cirrhosis and liver cancer. Although NAFLD is strongly associated with obesity and insulin resistance, its pathogenesis remains poorly understood, and therapeutic options are limited. Here, we discuss recent mechanistic insights into NAFLD, focusing primarily on those that have emerged from human genetic and metabolic studies.

Early in eukaryote evolution, triglycerides (TGs) emerged as the preferred storage nutrient to buffer against fluctuations in energy demand and availability. The ubiquitous selection of TG for this role is attributable to two physicochemical properties: TGs provide greater caloric density (9 kcal/g) than do carbohydrates (4.5 kcal/g) or proteins (4 kcal/g), and TGs are insoluble in water, so they can accumulate to high levels with no adverse osmotic or colloidal effects on cells. In higher organisms, TG is stockpiled in adipocytes, and it accumulates in other cell types only under unusual circumstances. For example, migratory birds store large quantities of TGs in the liver as an energy source in preparation for prolonged seasonal flights, a propensity that has been exploited to produce the culinary delicacy foie gras. Like migratory birds, some humans who consume excess calories deposit fat in the liver. In humans, however, fatty liver is maladaptive and can have severe clinical consequences.

Nonalcoholic fatty liver disease (NAFLD) is an umbrella term used to describe a range of related disorders (Fig. 1). The earliest stage is hepatic steatosis, which is characterized by the deposition of TG as lipid droplets in the cytoplasm of hepatocytes. Steatosis is defined as a hepatic TG level exceeding the 95th percentile for lean, healthy individuals (i.e., >55 mg per g of liver) or as the presence of cytoplasmic TG droplets in more than 5% of hepatocytes (Fig. 1B) (1). Hepatic steatosis is often self-limited, but it can progress to NASH (nonalcoholic steatohepatitis). NASH is distinguished from simple steatosis by the presence of hepatocyte injury (hepatocyte ballooning and cell death), an inflammatory infiltrate, and/or collagen deposition (fibrosis) (Fig. 1B). It is not known whether steatosis always

precedes NASH or whether steatosis and NASH are distinct disorders (Fig. 1A). NASH, in turn, can progress to cirrhosis: Between 10 to 29% of individuals with NASH develop cirrhosis within 10 years (2). In cirrhosis, hepatocytes are replaced by scar tissue composed primarily of type 1 collagen. The collagen is produced by specialized cells called stellate cells, which are activated by liver injury and play a key role in liver regeneration. Cirrhosis can ultimately progress to liver cancer (Fig. 1A); 4 to 27% of individuals with NASH-induced cirrhosis develop hepatocellular carcinoma (3). Hepatic TG content can be quantified by noninvasive imaging modalities, but liver biopsies are required to determine the stage of NAFLD.

The factors that promote TG deposition in the liver and the transition from steatosis to steatohepatitis and cirrhosis in humans have not been clearly defined. Mouse models that recapitulate certain features of the human disease continuum have provided insights into possible pathological mechanisms contributing to its development (4), but the relative roles of these pathways in humans have not been conclusively determined. In this review, we focus primarily on new insights that have emerged from human genetic studies. The recent identification of sequence variations that are associated with the full spectrum of NAFLD is likely to provide new molecular clues to the pathogenesis of this increasingly common disorder (5, 6).

The Pathogenesis of Hepatic Steatosis in Humans

Hepatic steatosis arises from an imbalance between TG acquisition and removal. TGs are assembled by coupling three fatty acids to a glycerol backbone via ester bonds. As shown in Fig. 2, the fatty acids used for hepatic TG formation are derived from three sources: (i) diet, (ii) de novo synthesis, and (iii) adipose tissue. Dietary fats taken up in the intestine are packaged into TG-rich chylomicrons and delivered to the systemic circulation. In rats, ~80% of the TG in chylomicrons is hydrolyzed by lipoprotein lipase (LPL), releasing free fatty acids (FFAs) for uptake by peripheral tissues. The remaining ~20% is deli-

vered to the liver (7). Extrapolating from these experiments, a typical American diet (100 g of fat per day) furnishes the liver with ~20 g of fat each day, equivalent to one-half of the total TG content of an average liver.

Carbohydrate feeding promotes de novo synthesis of FFA from acetyl-coenzyme A (CoA) (Fig. 2) by increasing the level of insulin and the availability of substrate. Insulin stimulates the transcription factor sterol regulatory element-binding protein-1c (SREBP-1c) via a signaling cascade involving AKT2, LXR, and mTOR (8). SREBP-1c up-regulates the enzymes that catalyze lipogenesis (9). Glucose also promotes lipogenesis by activating the transcription factor carbohydrate responsive element-binding protein (ChREBP) (10). Like SREBP-1c, ChREBP stimulates the expression of multiple genes in the fatty acid biosynthetic pathway. In addition, ChREBP increases expression of liver-type pyruvate kinase, thus providing more substrate for FFA and TG synthesis (10).

During fasting, plasma levels of insulin fall, whereas levels of glucagon and epinephrine increase, stimulating TG hydrolysis in adipocytes (Fig. 2). The first step in TG hydrolysis is catalyzed by adipocyte TG hydrolase (ATGL) (11). FFAs are released and transported to liver, mostly bound to albumin. FFAs in liver have three major fates: They can be oxidized in mitochondria to produce energy and ketone bodies, reesterified to TG and stored in lipid droplets, or coupled to apolipoproteins and secreted as a constituent of very-low-density lipoproteins (VLDL). FFAs in liver are also incorporated into phospholipids and other lipids. The flux of FFA through the circulation amounts to ~100 g/day with 20% being extracted by the liver. Thus, the daily input of TG from the diet (~20 g/day) and FFA from adipose tissue (~20 g/day) approximates the entire TG content of the liver.

Studies of humans with rare inherited disorders demonstrate that hepatic TG accretion from increased dietary intake, repartitioning of TG from adipose tissue to the liver, or increased de novo lipogenesis results in hepatic steatosis (12). Congenital generalized lipodystrophy is invariably associated with severe hepatic steatosis (12). Glycogen storage disease type 1a and citrin deficiency both lead to enhanced de novo lipogenesis and cause massive hepatic steatosis even in the absence of obesity or insulin resistance (13, 14). Citrin deficiency is caused by mutations in the mitochondrial aspartate-glutamate transporter. Inactivation of this transporter accelerates malate-citrate exchange, resulting in an increase in cytoplasmic citrate, which is converted to acetyl-CoA.

Genetic defects that prevent the removal of TG from the liver also cause steatosis. Mutations in ATGL or its cofactor, comparative gene identification-58 (CGI-58), prevent mobilization of FFA from lipid droplets (Fig. 2). Defects in the enzymes required for oxidation of FFA in mitochondria (the hydroxyacyl-CoA transferases)

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also cause hepatic steatosis (12). The major export pathway for hepatic TGs is secretion into the blood as VLDL (Fig. 2). Mutations in the structural protein of VLDL [apolipoprotein (apo)B] or in the protein that adds TG to the nascent lipoprotein particle in the endoplasmic reticulum (ER) (microsomal TG transfer protein, MTP) are additional causes of hepatic steatosis. Individuals heterozygous for inactivating mutations in *APOB* produce fewer VLDL particles and have a three-fold increase in hepatic TG relative to healthy individuals (15).

Whereas single-gene mutations cause rare forms of severe steatosis, the increased prevalence of NAFLD in the general population over the past 30 years is due to changes in the quantity and composition of food (Fig. 2). The cardinal role of obesity in the development of hepatic steatosis is evident from cross-sectional correlations. For example, in the Dallas Heart Study, a multiethnic population-based sample that included more than 2000 individuals from Dallas, Texas, hepatic steatosis is uncommon among lean individuals [9% in individuals with body mass index (BMI) < 25 kg/m²] and highly prevalent among the severely obese (51% in individuals with BMI > 35 kg/m²) (16). Food composition also influences hepatic fat deposition. Carbohydrates in general and fructose in particular play important roles. Fructose consumption routes dietary carbons directly to the liver in a form that is primed to enter biosynthetic pathways, including de novo lipogenesis. Unlike glucose, circulating fructose is taken up almost entirely by liver (17). Because fructose is phosphorylated at carbon 1 rather than carbon 6, it cannot be used to

also cause hepatic steatosis (12). The major export pathway for hepatic TGs is secretion into the blood as VLDL (Fig. 2). Mutations in the structural protein of VLDL [apolipoprotein (apo)B] or in the protein that adds TG to the nascent lipoprotein particle in the endoplasmic reticulum (ER) (microsomal TG transfer protein, MTP) are additional causes of hepatic steatosis. Individuals heterozygous for inactivating mutations in *APOB* produce fewer VLDL particles and have a three-fold increase in hepatic TG relative to healthy individuals (15).

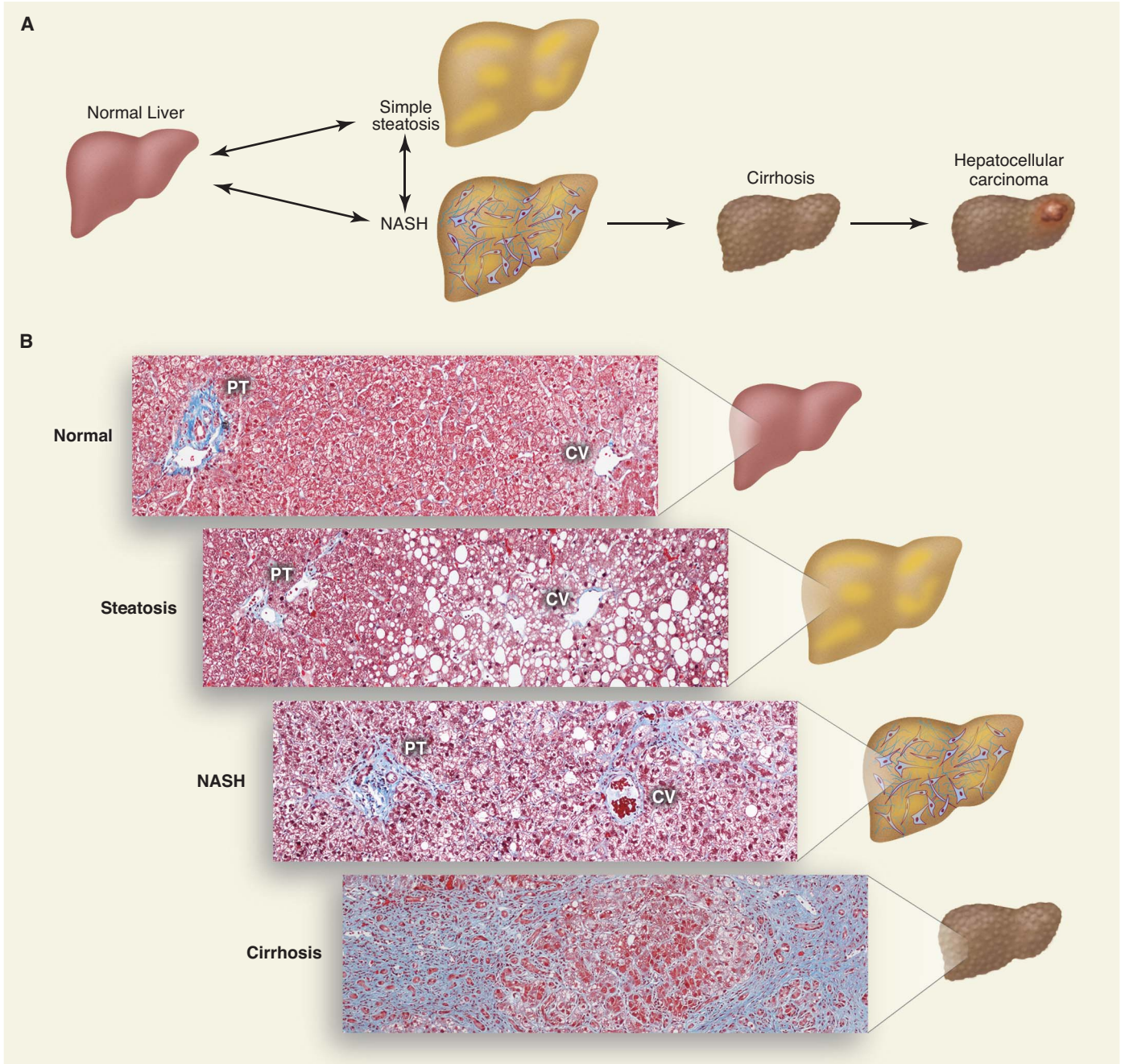


Fig. 1. The disease spectrum of nonalcoholic fatty liver disease. **(A)** Schematic of progression of NAFLD. The accumulation of TG within lipid droplets in hepatocytes causes steatosis. Steatosis associated with inflammation, cell death, and fibrosis is referred to as NASH, which can progress to cirrhosis. Individuals with

cirrhosis have an increased risk of hepatocellular carcinoma. **(B)** Histological sections illustrating normal liver, steatosis, NASH, and cirrhosis. Collagen fibers are stained blue with Masson's trichrome stain. The portal triad (PT), which consists of the hepatic artery, portal vein, and bile duct, and the central vein (CV) are shown.

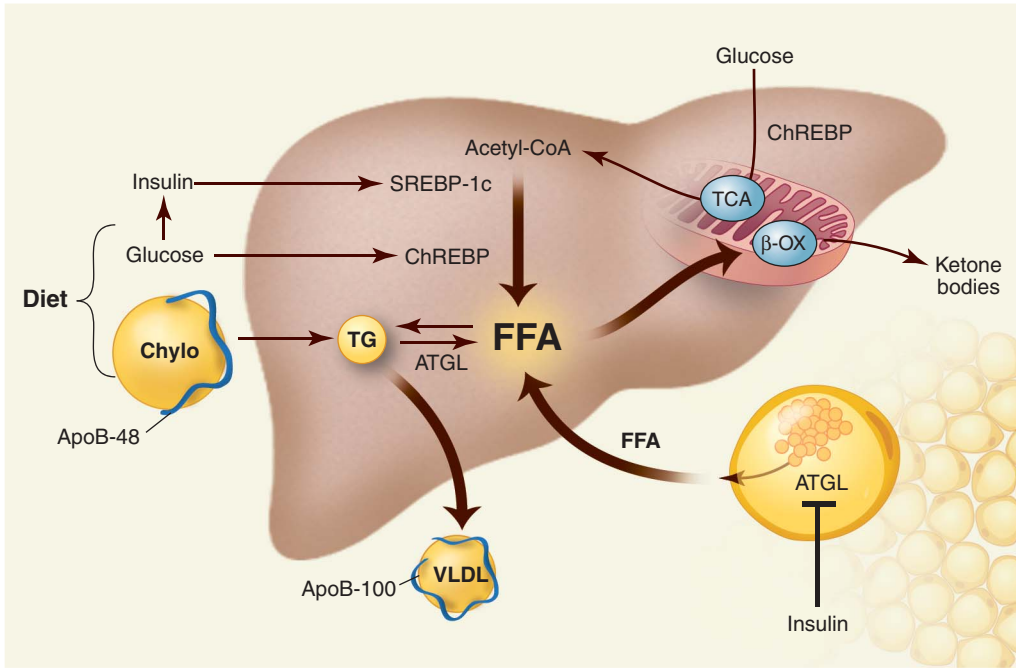


Fig. 2. Metabolism of TG in the liver. The three major sources of FFAs are diet, endogenous synthesis, and peripheral tissues. FFAs have four possible fates. They can be metabolized by β oxidation (β -OX) in mitochondria, esterified and stored as TG in lipid droplets, used to form other lipids (not shown), or packaged with apoB into VLDL and secreted into blood. Processes that increase FFA and TG input or reduce FFA and TG output cause hepatic steatosis. Carbohydrate intake increases glucose and insulin levels, which activate two transcription factors in the liver that promote de novo lipogenesis: ChREBP and SREBP-1c. Insulin inhibits lipolysis in adipose tissue by suppressing ATGL. Chylo, chylomicron; TCA, tricarboxylic acid.

synthesize glycogen but instead is quantitatively converted to glyceraldehyde-3-phosphate, providing substrate for de novo lipogenesis. The average yearly consumption of fructose has progressively increased and likely contributes to the increasing prevalence of NAFLD.

What Is the Relationship Between Obesity, Insulin Resistance, and Hepatic Steatosis?

The finding that genetic diseases that promote flux of energy substrates to fatty acids, such as glycogen storage disease type 1a and citrin deficiency, cause steatosis even in the absence of insulin resistance indicates that increased flux of FFA to the liver is sufficient to cause steatosis (13, 14). In obese individuals, the increased supply of FFA to the liver from the diet, from adipose tissue, and through increased de novo lipogenesis all serve to promote hepatic steatosis. The relative contributions of the three pathways to hepatic steatosis in humans have been defined in only one study: Donnelly *et al.* (18) reported that 59% of hepatic fat is derived from circulating FFAs, with lesser contributions from de novo lipogenesis (26%) and diet (15%).

A major unresolved question is whether NAFLD is a cause or consequence of insulin resistance. In liver, insulin inhibits glucose production and promotes fatty acid synthesis. With the development of hepatic insulin resistance, the inhibitory effect of insulin on glucose production is diminished, whereas the stimulatory effect of insulin on lipogen-

esis is retained (19). Insulin resistance is strongly correlated with steatosis, and interventions that ameliorate insulin resistance lead to lower insulin levels and decreased liver fat content. Multiple animal models support a direct causal relationship between insulin resistance, hyperinsulinemia, and hepatic steatosis (4). Evidence that insulin resistance causes steatosis in humans derives from patients with mutations in *AKT2* (20). These patients have profound resistance to the glucoregulatory actions of insulin but presumably retain sensitivity to the lipogenic effects of the hormone. Studies of metastatic insulin-secreting tumors (insulinomas) and from pancreatic islet cell transplants provide further evidence that insulin directly promotes fat accumulation in liver cells. Hepatocytes surrounding metastatic insulinomas become engorged with TGs, as do hepatocytes surrounding transplanted islet cells (21).

The coincident occurrence of hepatic steatosis and insulin resistance has led to the hypothesis that excess TG in liver causes insulin resistance. Hepatic steatosis and insulin resistance occur together in several strains of genetically modified mice (4, 22). However, the notion that hepatic steatosis causes insulin resistance is contradicted by observations in mice with defects in diverse pathways that cause hepatic steatosis without insulin resistance. Mice with reduced fatty acid synthesis (23), mobilization (24), or oxidation (4), as well as defective cytokine signaling (25) or choline synthesis (26), maintain normal or improved

insulin sensitivity despite hepatic TG accumulation. TG may be a marker for another molecule that interferes with insulin action, such as diacylglycerol (DAG), long-chain acyl-CoA, or ceramide. However, hepatic accumulation of any of these lipids does not invariably produce insulin resistance, at least in mice [for review, see (22)]. It remains possible that these lipids contribute to insulin resistance only if they accumulate within specific subcellular compartments or if they have a particular complement of fatty acids.

In humans, naturally occurring mutations provide a powerful tool to untangle mechanistic relationships between highly correlated metabolic traits. If increased hepatic TG content causes insulin resistance, then individuals with genetic variants that promote hepatic steatosis should be at increased risk of developing insulin resistance. An increasing number of Mendelian genetic defects have uncoupled these two variables. Individuals with inactivating mutations in *APOB* have increased levels of hepatic TG yet maintain normal insulin sensitivity (15). Patients with autosomal recessive disorders caused by mutations in either *ATGL* or *CGI58*

have severe steatosis but are not insulin resistant [reviewed in (12)]. In population-based studies, a genetic variant in *PNPLA3* that is associated with hepatic steatosis is not associated with insulin resistance (see below) (5). Sequence variants in *APOC3* have been associated with both hepatic steatosis and insulin resistance (25), but this association was not observed in other independent populations (26, 27). Thus, the preponderance of evidence is not compatible with the hypothesis that TG accumulation in hepatocytes causes insulin resistance in humans.

Genetic Risk Factors for Hepatic Steatosis

Although obesity and insulin resistance are the most prevalent risk factors for NAFLD, hepatic fat content varies substantially among individuals with equivalent adiposity, indicating that other factors contribute to this condition. One of these factors is gender. Before age 60, men are significantly more likely to develop steatosis than are women (16), but at older ages the disorder is more prevalent in women. Reasons for this gender dimorphism are not known. Another factor is ethnicity. In the Dallas Heart Study, hepatic steatosis was found in 45% of Hispanics, 33% of individuals of European ancestry, and 24% of African Americans (16). The higher prevalence of hepatic steatosis in Hispanics is due in part to a higher prevalence of obesity and insulin resistance in this population, but the lower prevalence in African Americans cannot be explained by

ethnic differences in BMI, insulin resistance, ethanol ingestion, or medication use. Another ethnic group with an increased prevalence of hepatic steatosis is Asian Indians. A study of 482 lean young individuals revealed a twofold higher hepatic TG content in Asian Indians than men of European descent (28).

Hepatic steatosis, NASH, and cirrhosis cluster in families (29), with the heritability of NAFLD being estimated to be ~39% (30). One genetic variant that is consistently associated with NAFLD is a missense mutation [Ile¹⁴⁸→Met¹⁴⁸ (I148M)] in patatin-like phospholipase domain-containing (PNPLA) 3 gene *PNPLA3* (also called adiponutrin) (5). This variant was initially identified through an association study of 9299 nonsynonymous sequence variations, and the relationship with hepatic TG content has been confirmed in many independent studies [for review, see (31)]. The frequency of the susceptibility variant (PNPLA3-I148M) in ethnic groups mirrors the prevalence of NAFLD and accounts for ~70% of the differences in frequency of hepatic steatosis between Hispanics, African Americans, and individuals of European descent (5). Homozygotes for the risk allele in *PNPLA3* (MM) have a ~twofold higher hepatic TG content, although the magnitude of the effect is strongly influenced by adiposity and insulin sensitivity.

PNPLA3 is a member of the PNPLA family, most closely resembling ATGL (PNPLA2) (Fig. 2) (11). PNPLA3 is most highly expressed in adipose tissue and liver and is transcriptionally reg-

ulated by insulin through a signaling cascade that includes LXR and SREBP-1c; hepatic PNPLA3 mRNA levels are reduced to nearly undetectable levels during fasting and increase 80-fold with refeeding in mice (32). Over 90% of the PNPLA3 in hepatocytes is located in lipid droplets, which are specialized organelles that participate in protein partitioning, trafficking, and degradation [for review, see (33)].

The physiological role of PNPLA3 and the mechanism by which the I148M isoform causes fatty liver are not known. The purified protein has both TG hydrolase and transacylase activity (34, 35). The I148M substitution markedly reduces TG hydrolase activity in vitro (35), suggesting that the I148M substitution causes a loss of function. However, inactivation of *Pnpla3* in mice fails to increase hepatic TG content (36, 37), and adenoviral-mediated overexpression of PNPLA3-I148M in mouse liver causes an increase in hepatic TG content (35), which is more consistent with the I148M substitution conferring a gain of function. Additional studies will be required to determine the molecular mechanism by which variation in PNPLA3 confers susceptibility to NAFLD.

A recent genome-wide association study of hepatic steatosis in 7176 participants (6) revealed additional susceptibility loci for NAFLD. Surprisingly, none of the newly identified genomic intervals contained genes associated with rare Mendelian disorders of hepatic steatosis, such as *APOB*, *ATGL*, *CGI-58*, or genes associated with lipodystrophy. The allele of greatest effect size

was PNPLA3-I148M, which conferred an odds ratio for NAFLD of 3.26. The other genomic regions associated with hepatic steatosis in this study included *NCAN* and *PPP1R3B* (Table 1). Analysis of an independent cohort with histologically defined NAFLD by the same group found an association with *NCAN*, *GCKR*, and *LYPLAL1* but not with *PPP1R3B*, with odds ratios ranging from 1.37 (*LYPLAL1*) to 1.65 (*NCAN*). Loss-of-function alleles in *GCKR* and *PPP1R3B* would be predicted to increase levels of glucose-6-phosphate and thus promote fatty acid synthesis. The mechanistic link between NAFLD and the other two implicated genes, *NCAN* and *LYPLAL1*, remains to be defined. Elucidation of the roles of these genes may provide new insights into the metabolic pathways that contribute to common forms of NAFLD in the population.

What Factors Contribute to NAFLD Progression?

The *PNPLA3* allele associated with steatosis is also associated with elevated serum levels of alanine transaminase (ALT) (5, 38), an enzyme released from injured hepatocytes, and with pathological features of NASH in biopsy samples (39). PNPLA3-I148M may also contribute more generally in the response of the liver to injury. In support of this notion, PNPLA3-I148M greatly increases the odds of developing cirrhosis in alcoholics (40). The finding that PNPLA3 is associated with hepatic steatosis, NASH, and cirrhosis provides strong molecular evidence that NAFLD is indeed a disease continuum as shown in Fig. 1.

Table 1. Common variants associated with nonalcoholic fatty liver disease. Odds ratios for NAFLD were calculated by using cases with biopsy-proven NAFLD and in ancestry-matched controls (6). EA, European American; AA, African American; ND, not determined.

Gene (first report)	Protein	Function	Allele variant	Minor allele frequency	Steatosis	NASH	Odds ratio NASH	Associations with other liver disorders
<i>PNPLA3</i> (5)	Patatin-like phospholipase domain-containing protein 3	TG hydrolase?	rs738409 I148M	0.23 in EA 0.49 in Hispanics 0.17 in AA	Yes	Yes	3.26	Alcohol-related cirrhosis Hepatocellular carcinoma
<i>PPP1R3B</i> (6)	Glycogen binding subunit of protein phosphatase 1	Enhances protein phosphatase 1 activation of glycogen synthase	rs4240624	0.08	Yes	No	0.93	ND
<i>NCAN</i> (6)	Neurocan	Unknown	rs2228603 P92S	0.07	Yes	Yes	1.65	ND
<i>GCKR</i> (6)	Glucokinase regulatory protein	Negative regulator of glucokinase	rs780094 P446L	0.39	No	Yes (not replicated)	1.45	ND
<i>LYPLAL1</i> (6)	Lysophospholipase-like 1	Unknown	rs12137855	0.21	No	Yes (not replicated)	1.37	ND
<i>APOC3</i> (25)	Apolipoprotein C3	Limits hydrolysis of TG in circulating lipoproteins	rs2854116 rs2854117	0.38 in EA 0.38 in Hispanics 0.71 in AA 0.26 in EA 0.32 in Hispanics 0.66 in AA	Yes*	ND	ND	ND

*The association was found in Asian-Indian men and replicated in a group of mixed ethnicity (25). The association was not observed in (26, 27).

About one-third of individuals with NAFLD who undergo liver biopsy have evidence of NASH. Of these individuals, 20 to 30% progress to severe fibrosis within 10 years (41). The factors causing progression of NAFLD to fibrosis and cirrhosis have not been defined in humans, in part because of the relative inaccessibility of liver tissue. Unfortunately, no animal model recapitulates the entire disease spectrum of NAFLD in humans (4). Nevertheless, studies in animal models have revealed several molecular processes that recapitulate the cardinal features of NASH: hepatocyte damage, inflammation, and fibrosis. Inflammation is a component of the wound healing process that leads to the deposition of extracellular matrix and fibrosis in liver. A growing body of evidence supports a central role for pro-inflammatory cytokines, particularly tumor necrosis factor α (TNF- α), and interleukin 6 (IL-6) (42) in the development of NASH. Levels of TNF- α and IL-6 are elevated in the liver and blood of patients with NASH, and inhibition of these cytokines has improved NAFLD in rodents (43). A second potential mechanism is ER stress, which results from improperly folded proteins accumulating in the ER, which elicits the unfolded protein response (UPR). The UPR activates nuclear factor κ B, c-Jun N-terminal kinase, and oxidative stress pathways, all of which have been implicated in progression of steatosis to NASH (44).

Extrahepatic factors may also contribute to NAFLD development and progression. Up to 70% of individuals undergoing liver transplantation for NASH or cryptogenic cirrhosis develop NAFLD in the transplanted liver, although the number of studies is small and factors such as immunosuppressive drugs may play a role (45). Evidence from mice suggest that increased exposure of hepatocytes to saturated fatty acids can trigger inflammation by interacting with Toll-like receptors and apoptosis by activating death receptors (46). Saturated fats can also inhibit mitochondrial function and induce the ER stress pathway in model organisms [see (46) for review].

Longitudinal studies of NAFLD show that a substantial number of individuals (up to 20 to 30%) improve between biopsies, suggesting that the disease might improve without treatment in some individuals (41). Striking reductions in hepatic TG content have been seen after bariatric surgery. A recent meta-analysis of 15 studies that included 766 paired liver biopsies revealed that bariatric surgery improved steatosis in 92% of patients, improved steatohepatitis in 81%, and led to complete resolution in 70% (47). Diet-induced weight loss with increased physical activity has also been shown to be associated with improvement in liver pathology (48). Recent studies using an antioxidant (vitamin E) and an insulin sensitizer, pioglitazone, have shown some success (49).

Outlook

NAFLD is a major health problem that has accompanied the trend toward an unhealthy diet. With recent advances in the prevention and treat-

ment of hepatitis C, NAFLD is poised to become the primary indication for liver transplantation. Of greatest concern is the increased prevalence of hepatic steatosis in children, where the disease may be more virulent (50). Despite the increasing morbidity associated with NAFLD, many questions remain regarding the pathogenesis, natural history, and treatment of this disorder. For example, why is the frequency of NAFLD lower in individuals of African descent even though the prevalence of obesity and insulin resistance is high in this group? Elucidating the molecular basis for these ethnic differences may provide new therapeutic targets for the treatment of NAFLD and other components of the metabolic syndrome.

A critical question is whether hepatic TG accumulation alone is sufficient to cause disease progression in NAFLD. Is the increase in NASH and cirrhosis associated with the PNPLA3 variant conferred solely by increasing liver TG content, or does the variant have other adverse effects? In the Dallas Heart Study, the effect of PNPLA3 on liver enzyme levels is abolished after controlling for liver TG content. Some patients with primary accumulation of hepatic TG, such as patients with homozygous familial hypobetalipoproteinemia or with neutral lipid storage disease, develop cirrhosis (12). However, these disorders are uncommon, and only a minority of patients progress to cirrhosis. Thus, it is not clear whether the liver disease in these individuals is due solely to the accumulation of TG in hepatocytes.

Improved methods for early detection of NASH are urgently needed. Hepatic steatosis can be diagnosed noninvasively, but determining the presence of hepatic inflammation and fibrosis requires a liver biopsy, which is usually reserved for individuals with elevated levels of circulating liver enzymes. Although having an elevated ALT increases the likelihood of having NASH, up to 59% of individuals with hepatic steatosis and normal ALTs have NASH on biopsy (51). Some patients with NAFLD present for the first time with cirrhosis. Development of noninvasive methods that detect inflammation and fibrosis in the liver are needed to more fully capture the natural history of this disorder and to test therapeutic approaches designed to halt or reverse disease progression. A further impediment toward the development of new therapeutic interventions is the lack of an animal model that fully recapitulates all the features of NAFLD (4). Until such a model becomes available, human genetic studies provide a good opportunity for delineating the molecular pathways that lead to steatosis, steatohepatitis, and cirrhosis.

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16-Month-Olds Rationally Infer Causes of Failed Actions

Hyowon Gweon and Laura Schulz

To achieve our goals, we need to solve a fundamental inference problem: We need to distinguish our influence on event outcomes from the impact of the outside world. The distinction between attributions to the self and the world has been critical in disciplines ranging from social psychology (1) to artificial intelligence (2). The problem becomes urgent when our actions fail to achieve expected outcomes. If we try to turn on a light and are left in the dark, did we do something wrong (e.g., flip the wrong switch), or is something wrong in the world (e.g., a bulb burned out)? These attributions have different implications for our subsequent actions. If we are the problem, we should change something about the agent (e.g., vary our actions or ask for help finding the switch); if the problem is external, we should try to change the world (or at least the light bulb). Consistent with empirical work showing that children draw accurate inductive inferences from minimal data (3, 4), we show that infants can use sparse evidence about the distribution of failed outcomes to answer the question, “Is it me or the world?”

In experiment 1 (Fig. 1), infants were seated next to a parent and shown toys that differed only in color (green, yellow, and red). The experimenter pushed a button on the green toy, and the toy played music. She placed the red toy on a cloth near the infant and handed the infant either the green (within-object condition) or the yellow (between-objects condition) toy. As expected (5), all children pressed the button and pressed equally often between conditions [$t(26) = 1.42$, $P = \text{not significant (ns)}$] (6). The toy never worked for the child.

To decide whether the problem lies with the agent or the object, infants should consider both the relative plausibility of the two hypotheses and the statistical evidence for each (7). In the within-object condition, neither hypothesis initially appears very probable: The infant might be doing something subtly wrong (e.g., not pressing hard enough), or something nonobvious might be wrong with the toy (e.g., it might have broken during the transfer). However, the statistical evidence favors the agent hypothesis: the outcome covaries with the agent independent of the object. By contrast, in the between-objects condition, the statistical evidence is uninformative: The outcome covaries with both the agent and the object. Here, however, the object hypothesis is the more plausible on prior

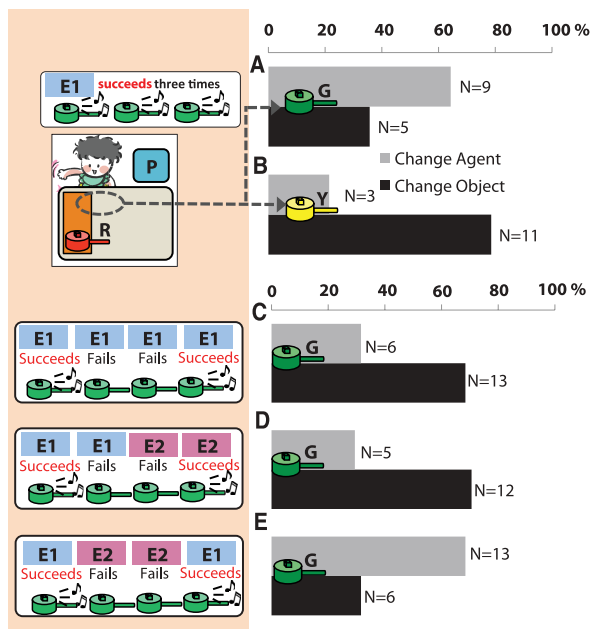


Fig. 1. Design and results. Experiments 1 [(A) within-object and (B) between-objects] and 2 [(C) within-agent 1, (D) within-agent 2, and (E) between-agents]. P indicates parent; E1 and E2, experimenters 1 and 2; G, Y, and R refer to toy colors: green, yellow, and red. The toy on the graph indicates the toy handed to the infant.

grounds: Although the infant's actions are not obviously different from the experimenter's, the toy clearly is. Moreover, there are now many ways the toy might have failed (e.g., the yellow toy might have broken at any point, or yellow toys might never work). As predicted, infants were more likely to try to change the agent (by handing the toy to their parents) than the object (by pulling the cloth or pointing to get the red toy) in the within- than between-objects condition (all P values ≤ 0.05 by Fisher's exact test: change agent versus change object, within-object, 64.3% versus 35.7%; between-objects, 21.4% versus 78.6%).

These results suggest that infants rationally use sparse data to make causal attributions. However, other interpretations are possible. Infants who received the experimenter's toy might have been less likely to want a new toy than those who did not. Alternatively, infants in the within-object condition might have asked for help not because they attributed failure to themselves but because they inferred that the toy was broken and wanted the parent to fix it.

Experiment 2 addressed these possibilities. Infants were assigned to one of three conditions, identical to the within-object condition except as follows: within-agent 1, a single experimenter suc-

cessfully activated the green toy twice and failed twice; within-agent 2, two experimenters each activated the green toy once and failed once (8); or between-agents, one experimenter activated the green toy twice and another experimenter failed twice. Children pressed the button equally often across conditions [$F(2,51) = 0.59$, $P = \text{ns}$], and the toy never activated.

These conditions differ only with respect to the statistical evidence. The outcomes in the within-agent conditions (considering also the infant's failure) vary independent of the agent, suggesting the failure is due to the object; the outcomes in the between-agents condition covary with the agent, independent of the object, suggesting the failure is due to the agent. As predicted, infants were more likely to first change the agent than the object in the between-agents than within-agent conditions (change agent versus change object, within-agent 1, 31.6% versus 68.4%; within-agent 2, 29.4% versus 71.6%; between-agents, 68.4% versus 31.6%).

Consistent with formal models of causal induction (7), these results suggest that infants track the statistical dependence between agents, objects, and outcomes and can use minimal data to draw inferences that support rational action. When the infants inferred that they were the source of failure, they sought help; when they believed the failure was due to their object, they explored others. Seeking instruction and engaging in exploration are both potentially effective strategies for learning; infants' differential response to failure depending on the evidence for its causes augurs well for their success.

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Scale for the Phase Diagram of Quantum Chromodynamics

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Matter described by quantum chromodynamics (QCD), the theory of strong interactions, may undergo phase transitions when its temperature and the chemical potentials are varied. QCD at finite temperature is studied in the laboratory by colliding heavy ions at varying beam energies. We present a test of QCD in the nonperturbative domain through a comparison of thermodynamic fluctuations predicted in lattice computations with the experimental data of baryon number distributions in high-energy heavy ion collisions. This study provides evidence for thermalization in these collisions and allows us to find the crossover temperature between normal nuclear matter and a deconfined phase called the quark gluon plasma. This value allows us to set a scale for the phase diagram of QCD.

Quantum chromodynamics (QCD) is the theory of strong interactions—one of the four fundamental interactions occurring in nature and an essential part of the standard model of particle physics. It describes interactions between quarks and gluons, which are the ultimate constituents of the majority of the visible mass of the universe (1, 2). In the short-distance regime in which the momentum exchange between quarks and gluons is large, the strong coupling constant becomes small through the mechanism of asymptotic freedom. In this perturbative region, QCD is very successful in explaining various processes observed in experiments involving electron-positron, proton-proton, and proton-antiproton collisions (3). In the non-perturbative regime, tests of the theory were related to the computation of hadron properties (4). In other regimes of long-distance nonperturbative physics, the theory is yet to be tested. Here, we test the thermodynamics of bulk strongly interacting matter.

Experimental tests of nonperturbative QCD in the bulk can be carried out by colliding heavy ions (such as U, Pb, Au, and Cu) at different center-of-mass energies, $\sqrt{s_{NN}}$ (5–8). Several experimental programs have been launched or are in the planning stage at facilities such as the Large Hadron Collider (LHC), the Relativistic Heavy Ion Collider (RHIC), the Super Proton Synchrotron (SPS), the Facility for Anti-proton and Ion Research (FAIR), and the Nuclotron-based Ion Collider fAcility (NICA), where the

essential features of the QCD phase diagram can be studied.

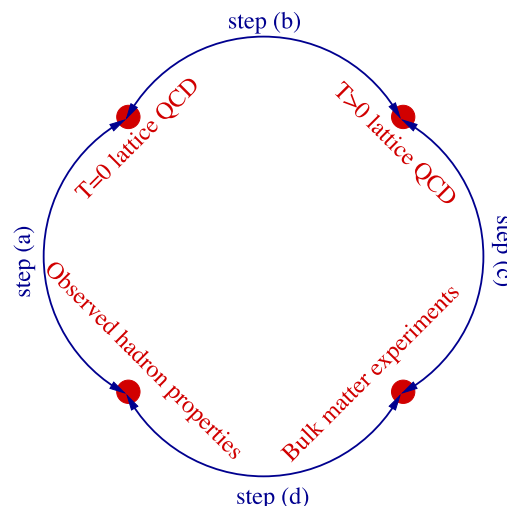
In QCD, there are conserved quantities such as the net-baryon number B , the net-electric charge Q , and the net-strangeness S . The term “net” means the algebraic sum of the quantum numbers, where those of anti-particles are the negatives of the corresponding particles. As a result, the thermodynamics of the bulk can be characterized by the corresponding chemical potentials (energy needed to add or remove one unit of the conserved quantity to or from the system, respectively) μ_B , μ_Q , and μ_S in addition to the temperature T , conjugate to the conserved energy of a bulk system. In experimental studies of particle ratios measured in heavy-ion collisions, it is observed that the relevant values of μ_Q and μ_S are small compared with μ_B . For example, in Au ion collisions within rapidity range of ± 0.1 unit at $\sqrt{s_{NN}} = 200$ GeV (with impact parameter less than 3 fm) one finds that $\mu_B = 22 \pm 4.5$ MeV, whereas $\mu_S = 3.9 \pm 2.6$ MeV, and μ_Q is still smaller (9).

The lattice formulation of QCD is a non-perturbative approach from first principles for

obtaining the predictions of QCD. Space-time is replaced by a lattice; quarks occupy the sites, and gluons occupy the links between the sites. The lattice spacing a is the inverse of the cutoff required to regulate any interacting quantum field theory. The theory is solved numerically at several values of a . The extrapolation to the continuum ($a = 0$) can then be made through the renormalization group equations. In QCD, there is a conventional temperature, T_c , that is an intrinsic scale of bulk hadronic matter. We follow the definition that it is the temperature at the peak of a susceptibility related to the confinement-deconfinement order parameter (called the Polyakov loop susceptibility, χ_L) at $\mu_B = 0$ (10–13). Lattice QCD computations show that this peak is finite, which corresponds to a crossover (14, 15). The temperature at which χ_L peaks, of course, changes with μ_B . However, once T_c is known such shifts as a function of μ_B can be quantified. This is similar to saying that the Celsius scale of temperature is defined by the boiling point of water at normal pressure P , and that the boiling point changes with P .

One of the most basic questions to ask about bulk hadronic matter is the value of T_c . This can be represented as a link in a “circle of reasoning” that encompasses all the regimes of nonperturbative QCD (Fig. 1). So far, the strategy to find T_c has been indirect: First, lattice QCD computations are performed at both $T = 0$ and $T > 0$ in order to determine a ratio T_c/m , where m is a typical hadronic scale [Fig. 1, step (b)]. Then one replaces the scale m , determined on the lattice, with an experimental measurement [Fig. 1, step (a)]. The temperature at each $\sqrt{s_{NN}}$ extracted from models of particle yields (16, 17) is step (d) of the circle of reasoning. From such models, one finds that the fireball of bulk nuclear matter created in heavy ion collisions, which is initially out of equilibrium, evolves to a state of thermal equilibrium at chemical freeze-out. The models do not give T_c ; however, they allow the extraction of T and μ_B at freeze-out. We show that predictions

Fig. 1. Illustration of the chain of reasoning for testing QCD in the nonperturbative domains of the strong interactions and obtaining the scale, T_c , of the QCD phase diagram.



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of lattice computations of finite temperature QCD (18), taken in conjunction with determinations of T_c in step (b) (10–13), agree well with experimental measurements on bulk hadronic matter (19). This allows us to invert the reasoning and extract T_c directly from the experimental measurements in heavy ion collisions [Fig. 1, step (c)]. The agreement of the temperature from steps (c) and (d) along with the agreement of T_c extracted from steps (a) and (b) with that from (c) show the complete compatibility of a single theory of hadron properties and of bulk QCD matter, that is, of all nonperturbative regimes of the strong interactions. This approach may present a new domain of tests of the standard model of particle physics.

The conjectured phase diagram of QCD. In the current conjectures for the parts of the phase diagram that is accessible with heavy ion collisions (Fig. 2) (20), calculations within simplified models that mimic QCD show that at large μ_B there is a first-order hadron–quark–gluon plasma (QGP) phase transition. This phase boundary is expected to end in a critical point at finite μ_B because lattice computations (10–13) agree with general symmetry arguments (21), which indicate that at $\mu_B = 0$ there is neither a first-order nor a second-order phase transition but only a crossover at T_c . The determination of T_c sets the scale of the QCD phase diagram. Current best estimates of the position of the critical point (22) are reflected in the position indicated in Fig. 2. Currently, the experimental focus is on an attempt to locate the critical point and the line of phase coexistence (23, 24).

By changing $\sqrt{s_{NN}}$, one traces out a line of chemical freeze-out in the phase diagram, as shown in Fig. 2. This line is parameterized through a hadron resonance gas model (16, 17). Because this work focuses on making a connection between QCD thermodynamic calculations and observables measured in experimental facilities, we also show in Fig. 2 the range of μ_B values covered by various experiments as one traverses the chemical freeze-out line by changing $\sqrt{s_{NN}}$. The solid point around $\mu_B = 938$ MeV is the location of ordinary nuclear matter (25), the best characterized point on the phase diagram.

Comparison of experimental measurements with lattice QCD predictions. Lattice QCD computations leave open the question of a scale and yield dimensionless predictions—for example, for P/T^4 as a function of T/T_c and μ_B/T . Here, we discuss the nonlinear susceptibilities (NLSs) of baryons, $\chi_B^{(n)}$, of order n (26). These are the Taylor coefficients in the expansion of P with respect to μ_B at fixed T in the usual dimensionless form

$$T^{n-4} \chi_B^{(n)} \left(\frac{T}{T_c}, \frac{\mu_B}{T} \right) = \frac{1}{T^4} \frac{\partial^n}{\partial (\mu_B/T)^n} P \left(\frac{T}{T_c}, \frac{\mu_B}{T} \right) \bigg|_{T/T_c} \quad (1)$$

Lattice measurements of the series expansion of the NLS in powers of μ_B/T are resummed by

using Padé approximants in order to give predictions for the above quantities (18). They are of interest because they are related to cumulants of the fluctuations of the baryon number in thermal and chemical equilibrium in a grand canonical ensemble.

The n th cumulant of such fluctuations, $[B^n]$, is given by

$$[B^n] = VT^3 T^{n-4} \chi_B^{(n)} \left(\frac{T}{T_c}, \frac{\mu_B}{T} \right) \quad (2)$$

where V is the volume of the observed part of the fireball. Because observed hadrons are in thermal and chemical equilibrium at the freeze-out, this relation should hold for cumulants of the observed event-by-event distribution of net-baryon number in heavy ion collisions. The cumulants are often reported as the variance $\sigma^2 = [B^2]$,

Fig. 2. Current conjectures for the QCD phase diagram. The phase boundary (solid line) between the normal low-temperature hadronic phase of bulk QCD matter and the high-temperature partonic phase is a line of first-order phase transitions that begins at large μ_B and small T and curves toward smaller μ_B and larger T . This line ends at the QCD critical point, whose probable position, derived from lattice computations, is marked by a square. At even smaller μ_B , there are no phase transitions, only a line of cross-overs (dashed line). The red-yellow dotted line corresponds to the chemical freeze-out line from the evolution of the bulk QCD matter produced in high-energy heavy-ion collisions. The solid point at $T = 0$ and $\mu_B = 938$ MeV represents nuclear matter in the ground state. At large μ_B and low T is the color superconductor phase (CSC) (35).

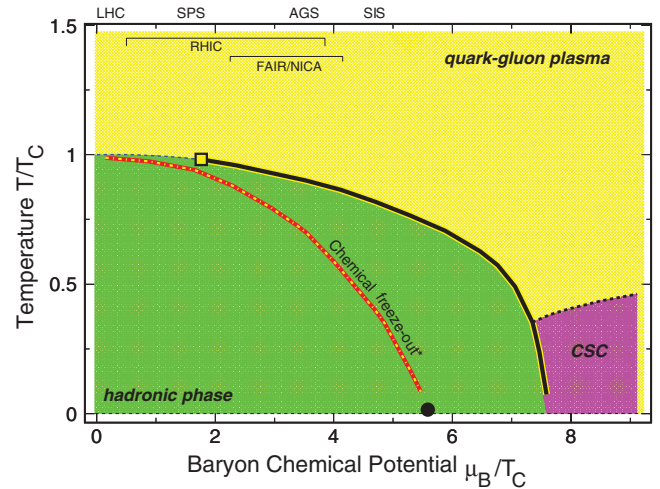
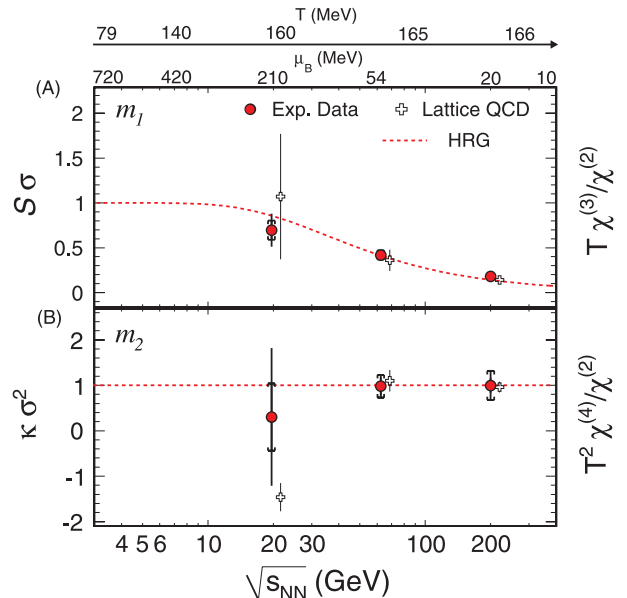


Fig. 3. Comparison of lattice QCD and experimental data for (A) m_1 and (B) m_2 . Experimentally measured ratios of cumulants of net-proton distributions, $m_1 = S\sigma$ and $m_2 = \kappa\sigma^2$, are shown as a function of $\sqrt{s_{NN}}$ for impact parameter values of less than 3 fm for Au+Au collisions at RHIC (19). Also plotted on the top scale are the chemical freeze-out values of μ_B and T corresponding to $\sqrt{s_{NN}}$ as obtained from a hadron resonance gas model, which assumes the system to be in chemical and thermal equilibrium at freeze-out (16, 17). The prediction of such a model for m_1 (33) is shown as the dashed red line. The lattice predictions for these quantities are drawn from a computation with lattice cutoff of $1/a \cong 960$ to 1000 MeV and converted to the dimensional scale of T and μ by using $T_c = 175$ MeV.



of fluctuations. However, the effect of isospin fluctuations on the shape of the net-baryon distributions is small (29). Hence, we proceeded under the assumption that the shape of the net-proton distributions reflects the net-baryon distributions up to distortions smaller than the estimated errors in measurements of the cumulants.

We are unable to exploit Eq. 2 directly in heavy-ion experiments because the volume, V , is hard to determine precisely experimentally. However, the ratios

$$\begin{aligned} (m_1) : S\sigma &= \frac{[B^3]}{[B^2]} = \frac{T\chi_B^{(3)}}{\chi_B^{(2)}}, \\ (m_2) : \kappa\sigma^2 &= \frac{[B^4]}{[B^2]} = \frac{T^2\chi_B^{(4)}}{\chi_B^{(2)}}, \\ (m_3) : \frac{\kappa\sigma}{S} &= \frac{[B^4]}{[B^3]} = \frac{T\chi_B^{(4)}}{\chi_B^{(3)}} \end{aligned} \quad (3)$$

do not contain the volume and therefore provide a direct and convenient comparison of experiment and theory (30). The above equations are written in a form that emphasizes this connection: The left hand side can be measured in an experiment, whereas the right hand side can be predicted with lattice QCD. We use the notation $m_{1,2,3}$ generically to refer to either side.

We now discuss the comparison of m_1 and m_2 from experiment and theory (Fig. 3). The experimental measurements (19) were made by using the number of protons (p) and anti-protons ($-p$) produced in the collision of Au ions around 90° to the beam axis with the impact parameter of the collisions being less than 3 fm (31). p and $-p$ are in the range of 400 MeV/ c to 800 MeV/ c , where c is the speed of light. This choice of momentum range is designed to obtain the purest sample of p and $\bar{p}$. A large fraction of p and $\bar{p}$ is contained in this kinematic range. The effect of finite reconstruction efficiency of p and $\bar{p}$ has been shown to be negligible (19). The ex-

perimental values of $S\sigma$ and $\kappa\sigma^2$ are shown as a function of $\sqrt{s_{NN}}$.

The lattice calculations (18) were carried out by using two flavors of staggered quarks in QCD. The lattice cutoff $1/a \cong 960$ to 1000 MeV and the bare quark mass were tuned to give a pion mass of about 230 MeV (32). These computations were performed at $\mu_B = 0$, and the Taylor series coefficients of P/T^4 were used to extrapolate m_1 and m_2 to the freeze-out conditions by using appropriate order Padé approximants to resum the series expansions. Because lattice results are obtained in terms of T/T_c and μ_B/T (Eq. 1), their extrapolation to the freeze-out conditions required the input of T_c . The lattice values were obtained by using $T_c = 175$ MeV, which is compatible with indirect determinations of T_c (10–13).

On the upper scales of Fig. 3, we also show the μ_B and T values at chemical freeze-out that correspond to the various $\sqrt{s_{NN}}$. For this, we used the functional relationship between these values from the hadron resonance gas model using the yields of hadrons discussed in (16, 17). The model predictions for m_1 (33) are also shown. The hadron resonance gas model predictions can be reproduced if baryon and anti-baryon numbers are independently Poisson distributed. Having established a connection between $\sqrt{s_{NN}}$ and (T, μ_B) , we compare the experimental data on fluctuations with those predicted from lattice QCD. Excellent agreement is seen between lattice QCD predictions and experimental measurements for all three beam energies. This marks the first successful direct test of QCD against experimental data in the nonperturbative context of bulk hadronic matter. The agreement with the data are yet another nontrivial indication that the fireball produced in heavy ion collisions is in thermal and chemical equilibrium at chemical freeze-out.

Setting the scale of bulk QCD. Lattice QCD results for $m_{1,2,3}$ are obtained for dimensionless arguments T/T_c and μ_B/T , as shown in Eq. 2. For

a given value of $\sqrt{s_{NN}}$, the experimental observations are realized at the corresponding chemical freeze-out, characterized by T and μ_B . Thus, a comparison of the two requires a choice of the scale, T_c . By varying this scale to obtain the best fit between the QCD predictions and experimental measurements, we determined T_c for the first time through an observable connected to strongly interacting bulk matter. The results are, of course, subject to all the caveats expressed in the previous section. The observable that we choose for comparison is m_3 . The lattice computation of this quantity has the smallest systematic uncertainties among the three explored here and thus is the best quantity to use to constrain T_c .

The comparison of m_3 between experimental results from Au ion collisions and lattice QCD predictions is shown in Fig. 4A. This is an extension of Fig. 3, which shows a comparison with m_1 and m_2 . In this analysis, the results of m_1 , m_2 , and m_3 are consistent, as required in Eq. 3. The new information here is that we show lattice predictions obtained with different values of T_c . The errors on the experimental data points are statistical (lines) and systematic (brackets) errors (19). The errors bars on the lattice predictions are statistical errors, with a cutoff of $1/a \cong 960$ to 1000 MeV. The lattice spacing effects and the effect of tuning the bare quark mass are the main sources of remaining uncertainties in the predictions. These are not parameterized as systematic uncertainties. However, it is known that their effect is small at the two highest values of $\sqrt{s_{NN}}$ (18).

In order to arrive at a quantitative estimate of the scale parameter T_c , we perform a standard statistical analysis. For each value of T_c , we compute

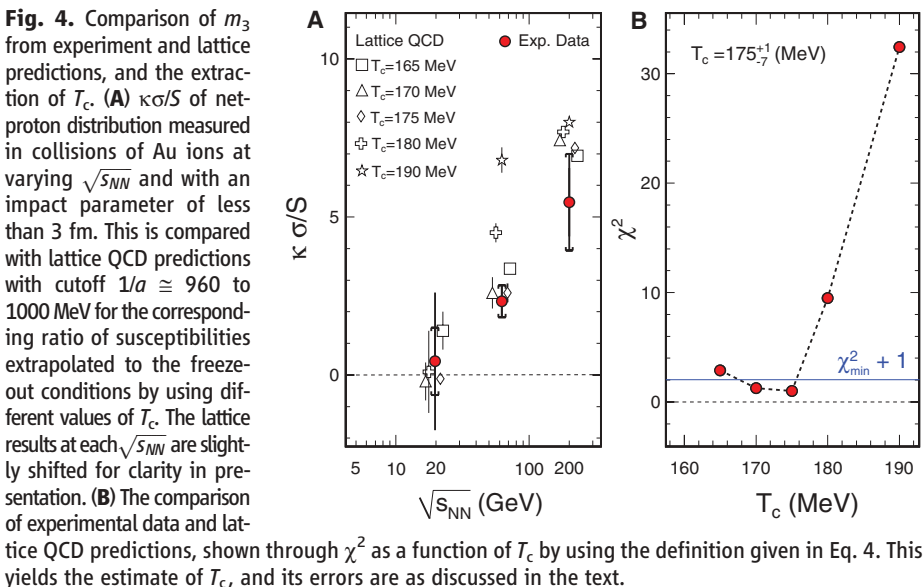
$$\chi^2(T_c) = \sum_{\sqrt{s_{NN}}} \frac{[m_3^{\text{expt}}(\sqrt{s_{NN}}) - m_3^{\text{QCD}}(\sqrt{s_{NN}}, T_c)]^2}{\text{Error}_{\text{expt}}^2 + \text{Error}_{\text{QCD}}^2} \quad (4)$$

where the errors in the experimental and lattice QCD quantities are obtained as explained above. The lattice predictions are calculated for the grid of T_c values (Fig. 4). The minimum of χ^2 , corresponding to the most probable value of the parameter being estimated, occurs at $T_c = 175$ MeV. The standard errors on the parameter are the values of T_c for which χ^2 exceeds the minimum value by unity. It is clear from Fig. 4B that this is bounded by +5 and -10 MeV. A piecewise linear interpolation between the grid points yields the more reliable error estimate, +1 and -7 MeV. By comparing different interpolation schemes, we found that the error estimate is stable. As a result, we conclude that

$$T_c = 175_{-7}^{+1} \text{ MeV}. \quad (5)$$

The error estimates include systematic and statistical errors on experimental data but only statistical errors on the lattice QCD computations.

The result in Eq. 5 is compatible with current indirect estimates of T_c that come from setting the



scale of thermal lattice QCD computations via hadronic observables. Furthermore, this gives a scale for temperatures that is compatible with the resonance gas model, as shown in Fig. 3. As we discussed in the introduction, this closes a circle of inferences that shows that phenomena obtained in heavy ion collisions are fully compatible with hadron phenomenology and provides a first check in bulk hot and dense matter for the standard model of particle physics.

Conclusions and outlook. We have performed a direct comparison between experimental data from high-energy heavy ion collisions on net-proton number distributions and lattice QCD calculations of net-baryon number susceptibilities. The agreement between experimental data, lattice calculations, and a hadron resonance gas model indicates that the system produced in heavy ion collisions attained thermalization during its evolution. The comparison further enables us to set the scale for nonperturbative, high-temperature lattice QCD by determining the critical temperature for the QCD phase transition to be 175^{+1}_{-7} MeV.

This work reveals the rich possibilities that exist for a comparative study between theory and experiment of QCD thermodynamics and phase structure. In particular, the current work can be extended to the search for a critical point. In a thermal system, the correlation length (ξ) diverges at the critical point. ξ is related to various moments of the distributions of conserved quantities, such as net-baryons, net-charge, and net-strangeness. Finite size and dynamical effects in heavy ion collisions put constraints on the values of ξ (34). The lattice calculations discussed here and several QCD-based models have shown that moments of net-baryon distributions are related to baryon number susceptibilities and that the ratio

of cumulants $m_2 = \kappa\sigma^2$, which is related to the ratio of fourth-order to second-order susceptibilities, shows a large deviation from unity near the critical point. Experimentally, $\kappa\sigma^2$ can be measured as a function of $\sqrt{s_{NN}}$ (or T and μ_B) in heavy ion collisions. A nonmonotonic variation of $\kappa\sigma^2$ as a function of $\sqrt{s_{NN}}$ would indicate that the system has evolved in the vicinity of the critical point and thus could be taken as evidence for the existence of a critical point in the QCD phase diagram.

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The Oxygen Isotopic Composition of the Sun Inferred from Captured Solar Wind

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All planetary materials sampled thus far vary in their relative abundance of the major isotope of oxygen, ^{16}O , such that it has not been possible to define a primordial solar system composition. We measured the oxygen isotopic composition of solar wind captured and returned to Earth by NASA's Genesis mission. Our results demonstrate that the Sun is highly enriched in ^{16}O relative to the Earth, Moon, Mars, and bulk meteorites. Because the solar photosphere preserves the average isotopic composition of the solar system for elements heavier than lithium, we conclude that essentially all rocky materials in the inner solar system were enriched in ^{17}O and ^{18}O , relative to ^{16}O , by $\sim 7\%$, probably via non-mass-dependent chemistry before accretion of the first planetesimals.

The gravitational collapse of a molecular cloud fragment 4.57 billion years ago led to an accretion disc of gas and dust, the

solar nebula, from which the Sun and planets formed. This nebula was approximately homogeneous with respect to isotopic abundances,

which, given that isotope ratios from various stellar nucleosynthetic processes vary widely, points to efficient mixing either in interstellar space or in the solar nebula. Thus, the discovery (1) that high-temperature minerals in carbonaceous chondrite meteorites are enriched preferentially in ^{16}O compared to ^{17}O and ^{18}O relative to the abundances in terrestrial samples was considered evidence for the presence of exotic material that escaped thorough mixing and thereby

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retained a memory of its distinct nucleosynthetic history. Later findings of the widespread nature of very large oxygen isotopic heterogeneities among meteoritic materials (2, 3) and the lack of correlation of these oxygen isotopic compositions with those of presolar components [e.g., (4, 5)] led to various proposals that the oxygen isotopic anomalies were instead generated by complex isotope-selective chemistry within a homogeneous nebula (6–10).

The nature of the processes, conditions, and events giving rise to oxygen isotope anomalies can be constrained with knowledge of the average initial oxygen isotopic composition of the solar nebula, which by mass balance is equivalent to the solar composition. Although oxygen is the third most abundant element composing the Sun (and the largest constituent of rocky planets), the solar oxygen isotopic composition has remained essentially unknown. The usual approach of inferring solar abundances from measurements of primitive meteorites is inadequate because of the aforementioned heterogeneities, and recent attempts to determine the Sun's oxygen isotopic composition from molecular absorption lines in the solar atmosphere are potentially prone to large systematic errors (11). To directly measure solar material, NASA flew the Genesis Discovery Mission to capture samples of the solar wind (SW) and return them to Earth for laboratory analysis (12).

Genesis orbited the Earth-Sun Lagrange point, L1, exposing collectors to the SW outside the Earth's magnetosphere between November 2001 and April 2004. In September 2004, the payload was returned to Earth; unfortunately, the parachute deployment mechanism failed and the sample return capsule struck Earth's surface at terminal velocity, shattering most of the target materials. In addition to passive collectors, Genesis included the Concentrator, a parabolic electrostatic mirror that reflected SW ions, focusing them onto backward-facing targets (13). Fortunately, these targets were suspended on a high-transparency wire mesh and survived the crash largely intact, suffering only some surface contamination. We selected an unbroken single-crystal SiC target (Fig. 1) for depth-profiling analysis by MegaSIMS, a hybrid secondary ion microscope–accelerator mass spectrometer (14) designed specifically to tackle the unique analytical challenges posed by the Genesis samples: dilute concentrations, limited sample material, and close proximity of surface contamination to the implanted SW ions. Here we report measurements of the oxygen isotopic composition of captured SW that demonstrate that the Earth and meteorites are grossly isotopically anomalous.

Isotopic measurement of captured solar wind. After inserting the sample into the analysis chamber of MegaSIMS (vacuum $<2 \times 10^{-11}$ Torr), we used reflected-light imaging to select areas that were free from defects or particles larger than a few micrometers. Even these apparently pristine areas were contaminated with oxygen adsorbed on the sample surface that ex-

ceeded the concentration of SW oxygen by orders of magnitude. We therefore cleaned the sample surface in situ by sputtering with a low-energy Cs beam rastered over $300 \mu\text{m}$ by $300 \mu\text{m}$ centered on the targeted area (Fig. 1) [supporting online material (SOM)]. Although the sputter cleaning removed the top ~ 20 nm of the sample, because of the low impact energy used (5 keV) only minimal amounts of surficial terrestrial oxygen were mixed into the implanted SW (14). After surface cleaning, we switched the MegaSIMS to analytical mode (20-keV impact energy, 20-nA Cs^+ rastered over $\sim 130 \mu\text{m}$ by $130 \mu\text{m}$) and used ion microscope imaging to briefly inspect the analytical area to ensure that it was free of contaminant dust particles larger than a few hundred nanometers. We employed frequent instrument baking and other measures to reduce levels of instrumental background to $<2\%$ of the SW fluence integrated over the top ~ 100 nm of the sample (SOM).

The MegaSIMS accelerated negative secondary ions to 10 keV, then passed them through an “isotope recombinator” mass filter (14) such that only masses 16 to 18 atomic mass units (amu) were injected into the tandem accelerator with its high-energy terminal held at +1.2 MV. Collisions with Ar gas stripped electrons from the incoming beam, destroying interfering molecular ions, including hydrides (14). We determined the in-depth distributions of implanted SW oxygen ions by simultaneous detection of the three O^{2+} isotope beams by ion counting (Fig. 2). To calibrate instrumental background, we continued sputtering through the implanted SW layer (peak

concentration depth ~ 80 nm) until a steady-state count rate was reached (typically at depths >300 nm). We corrected measured ion count rates for this background, for detector deadtime and duty cycle, and for mass-dependent isotopic fractionation in the MegaSIMS, which we determined by analysis of terrestrial standard materials under similar analytical conditions (SOM).

In principle, integration of the total counts in a depth profile yields the isotope ratios for the implanted SW. However, because of removal of the contaminated target surface, the integral cannot be taken over the entire SW profile. The implantation profiles (peak depth and width) are slightly different for each isotope as they depend on ion energy, and hence isotopic mass (15); accordingly, we fit a normal distribution to each isotope depth profile and summed the counts over the range from $-\sigma$ to 6σ relative to its peak depth. For a given depth profile, Poisson statistics limit the isotope ratio precision; analyses of oxygen-rich materials (terrestrial and meteoritic minerals) demonstrate accuracy of MegaSIMS to a level of $\sim 1\%$ (per mil).

The main purpose of the Concentrator was to increase both the effective fluence and implantation depth (16) of SW oxygen (and nitrogen) isotopes on the target collectors (13). The concentration factor is azimuthally symmetric, depending only on radial distance from the central axis of the Concentrator (17). However, because it must focus ions of different energies and different charge and mass, the Concentrator ion optics also impart a mass-dependent fractionation, which is a function of radial position on the

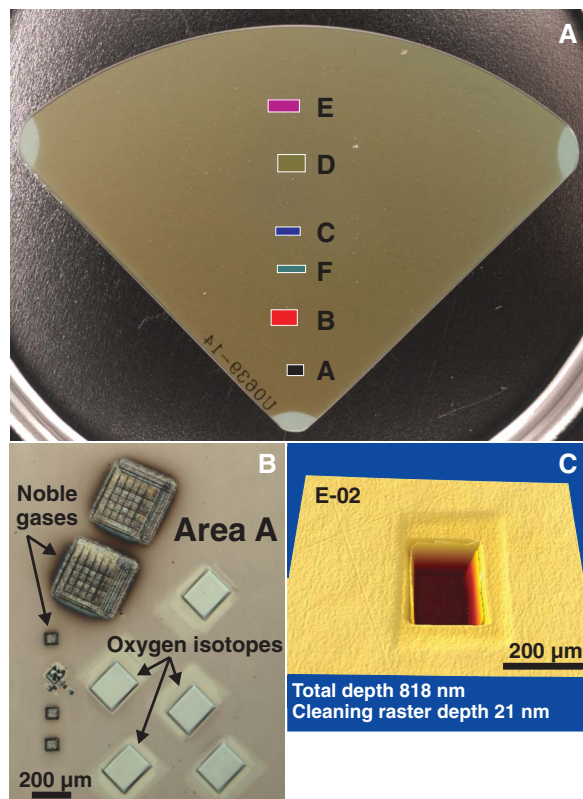


Fig. 1. (A) Photograph of Genesis SiC target 60001 (54) showing approximate areas analyzed along a radial traverse from inner region (area A) to periphery (area E) of the Concentrator. (B) Reflected-light micrograph of area A showing craters sputtered by Cs-beam rastering in MegaSIMS, along with two sets of pits made by laser ablation for noble gas analysis (17). The smaller pits were made to obtain $^{22}\text{Ne}/^{20}\text{Ne}$ data for correcting the mass-dependent fractionation induced at each radial position by the Concentrator ion optical system. (C) Rendering of surface topography made by an optical interferometer used to calibrate sputter rate. The effect of surface cleaning is visible as a shallow crater encompassing the area sampled by the deeper sputtered crater.

target. To assess the extent of concentration- and mass-dependent fractionation factors, we measured 40 depth profiles from 6 different areas in a traverse across the SiC sample (#60001) from 5.4 to 25.3 mm from the center (Fig. 1 and fig. S2). Neon isotopic compositions and fluence were later measured with high spatial resolution along the same radial traverse (17). The Ne isotope ratios of SW collected during the same period were previously determined in the bulk, passive SW collector, thereby permitting a direct measurement of the mass-dependent fractionation induced by the Concentrator as a function of target radius (17). We applied the Concentrator mass fractionation curve determined from $^{22}\text{Ne}/^{20}\text{Ne}$ to correct the $^{18}\text{O}/^{16}\text{O}$ and $^{17}\text{O}/^{16}\text{O}$ at each radial position (17) (SOM) (fig. S6).

From the interior (area “A”) to the exterior of the sample (area E, fig. S2), the implanted SW oxygen concentration decreases by a factor ~ 5 (fig. S3), consistent with the Ne traverse data (17). Of 40 depth profiles measured, 38 have integrated oxygen isotopic compositions that plot below the terrestrial mass fractionation line (TF), i.e., they are enriched in ^{16}O (SOM, table S1). Isotopic compositions from within the same area on the sample are reproducible, although the profiles from areas near the periphery of the target (areas D and E) show appreciable scatter because of poor counting statistics (fig. S5). The two profiles not enriched in ^{16}O come from area D and plot on the terrestrial mass fractionation line. The average compositions for each of the six areas analyzed (18) show clear evidence of mass-dependent fractionation, as expected on the basis of their radial positions on the Concentrator target (Fig. 3A). All analyses are consistent with an ^{16}O -rich composition for the captured SW with a mean $\Delta^{17}\text{O} = -28.4 \pm 1.8\text{‰}$ (Fig. 3C).

Correction of the measured $^{18}\text{O}/^{16}\text{O}$ and $^{17}\text{O}/^{16}\text{O}$ for Concentrator-induced mass-dependent fractionation as a function of radial position (17) (SOM) results in a convergence of all the data on a single composition within measurement uncertainties (Fig. 3B). Taking the average of the four areas measured within the interior 16 mm of the target (A, B, F, C), for which the concentration factors are highest ($>20\times$), we find that the SW collected by the Genesis spacecraft at L1 has a composition of $\delta^{18}\text{O} = -102.3 \pm 3.3\text{‰}$, $\delta^{17}\text{O} = -80.8 \pm 5.0\text{‰}$, where the uncertainty estimates are 1 standard deviation. In addition to being ^{16}O -enriched relative to terrestrial values, this composition is significantly to the left of the ^{16}O -mixing line defined by calcium-aluminum-rich inclusions (CAIs) of chondritic meteorites (19).

Inferring solar values from the solar wind.

In principle, the isotopic composition of SW oxygen collected at L1 could be equivalent to the photospheric value, but this is unlikely. First, spacecraft instruments (20, 21) and measurements on Genesis samples (22) detect enrichments of light isotopes in the low-speed relative to the fast SW, demonstrating that mass-dependent fractionation occurs in the acceleration of SW.

Both the sign (favoring light isotopes) and the magnitude—several percent for He (21, 22) and $<1\text{‰/amu}$ for heavier elements (22, 23)—are in approximate agreement with a model based on inefficient Coulomb drag (24). For a He/H ratio of 0.045 measured in the bulk SW on Genesis (25), this model predicts that the SW oxygen isotopic composition should be $\sim 26\text{‰/amu}$ lighter than the photosphere. Second, the existence of a SW-photosphere isotopic fractionation of this direction and magnitude is supported by the lack of meteoritic or planetary components with oxygen isotopic compositions plotting to the left of the CAI mixing line at the ^{16}O -enriched end (19, 26). Although many CAIs show mass-dependent fractionation favoring the heavy isotopes for non-refractory elements (e.g., Mg, Si), they rarely show mass fractionation effects in oxygen exceeding a few permil per amu. Those exceptions (i.e., the so-called FUN inclusions) have $\delta^{18}\text{O}$ values to the right of the CAI mixing line by tens of permil and are typically slightly less ^{16}O -enriched (i.e., less negative $\Delta^{17}\text{O}$) than our results for the SW (27).

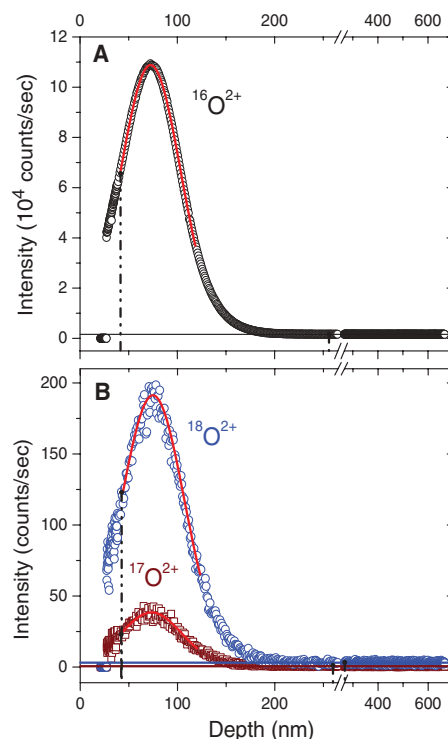


Fig. 2. In-depth distribution of implanted SW oxygen. Shown are measured ion intensity as a function of sputter depth for (A) $^{16}\text{O}^{2+}$ and (B) $^{17}\text{O}^{2+}$ and $^{18}\text{O}^{2+}$ in spot 4 of area A of the Genesis Concentrator SiC target 60001 and a normal distribution (red lines) fit to the peak of each profile. The top ~ 20 nm was removed by low-energy sputter cleaning; the first few measurement cycles have 0 counts because during this time the ion image was inspected for dust particles in the analytical area. Integration limits for each peak are indicated by vertical dashed lines (the small isotope specific differences are irresolvable at this scale). Note scale break at 265 nm. Background levels are indicated by horizontal lines.

These observations make it unlikely that the oxygen isotopic composition of CAIs resulted from mass-dependent fractionation from a solar composition equivalent to that measured for SW from L1. Given the consistent indications of fractionation from data in SW regimes, the model predictions, and expectations based on meteorite

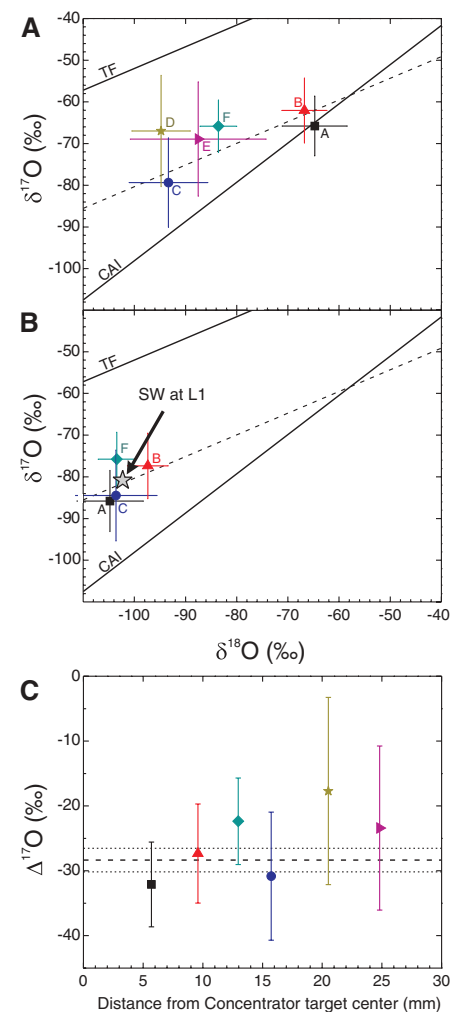


Fig. 3. (A) Mean oxygen isotopic compositions of SW from each group of depth profiles across a radial traverse of the Genesis Concentrator target. Shown are $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ corrected for instrumental mass fractionation in the MegaSIMS and normalized to standard mean ocean water (SMOW):

$$\delta^{18}\text{O} = \left(\frac{(^{18}\text{O}/^{16}\text{O})_{\text{sample}}}{(^{18}\text{O}/^{16}\text{O})_{\text{SMOW}}} - 1 \right) \times 1000, \text{ and similarly}$$

for $\delta^{17}\text{O}$. Shown for reference are the terrestrial mass-dependent fractionation line (TF) and the CAI mixing line (19). (B) SW oxygen isotope data from areas A, B, F, and C corrected for mass-dependent fractionation induced by the Concentrator (SOM). (C) The deviation from the terrestrial mass-dependent fractionation line expressed as $\Delta^{17}\text{O} = \delta^{17}\text{O} - (0.52 \times \delta^{18}\text{O})$ plotted as a function of radial position on the target. In (A) and (B), $\Delta^{17}\text{O}$ is the vertical displacement in $\delta^{17}\text{O}$ from a measured point to the TF line.

studies, we thus take as the most plausible composition of the Sun the intersection of the CAI mixing line with a mass-dependent fractionation line passing through the SW L1 point, which yields $\delta^{18}\text{O} = -58.5\text{‰}$, $\delta^{17}\text{O} = -59.1\text{‰}$ (Fig. 4). The total fractionation from SW to the Sun implied is $\sim 22\text{‰}$ /amu, in accordance with the model predictions.

The ^{16}O -enriched SW composition measured in the Genesis sample is in broad agreement with a component found from oxygen isotope measurements in the surface layers of some lunar metal grains (28) and of metal in a carbonaceous chondrite that shows evidence for SW exposure early in solar system history (29), although in both cases the data show a large degree of mass-dependent fractionation from the L1 SW value. Other analyses (30) of oxygen isotopes in a lunar regolith sample that indicate a ^{16}O -depleted composition ($\Delta^{17}\text{O} > 0$) probably reflect other extralunar sources, such as water brought by impacting interplanetary dust or cometary ices (31). Our measured SW composition is in marked contrast to the strongly ^{17}O - and ^{18}O -enriched values inferred from observations of rovibrational bands of CO in the solar atmosphere (32); we attribute this discrepancy to systematic uncertainties in the thermal profile models that underlie the abundance calculations (11). Our solar values of $^{16}\text{O}/^{18}\text{O} = 530$ and $^{16}\text{O}/^{17}\text{O} = 2798$ also disagree with other marginally ^{18}O -enriched values determined spectroscopically (33), although the data overlap within 2σ uncertainties.

The composition of the photosphere is thought to be representative of the convecting envelope of the Sun, representing $\sim 2.5\%$ of its

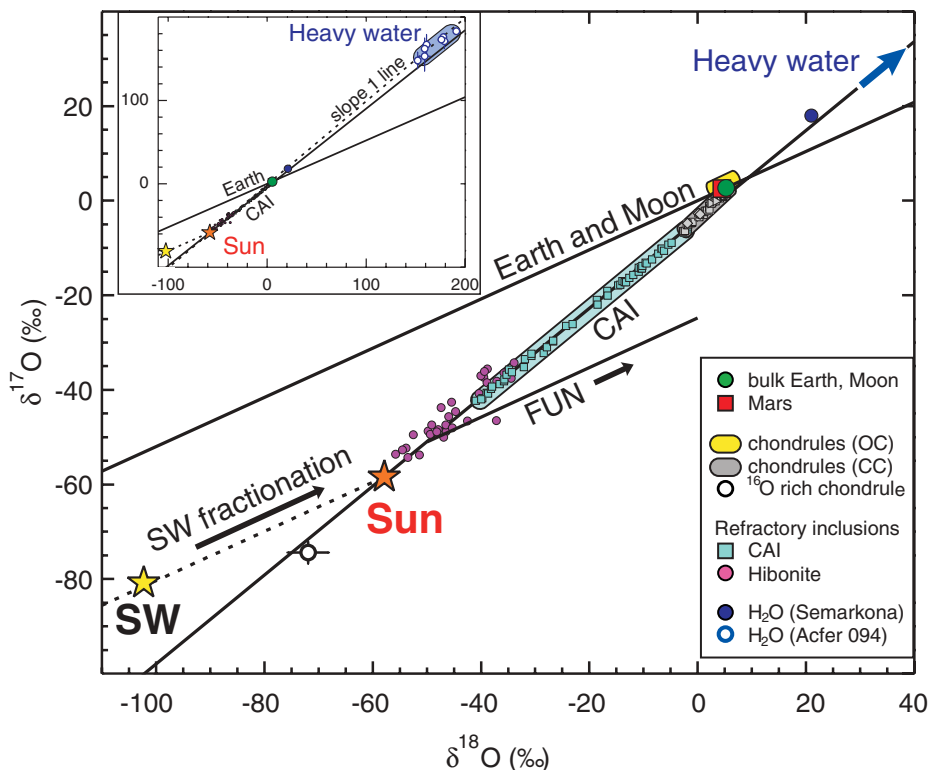
mass, perhaps modified slightly by gravitational settling of heavier elements [see (11)]. Although not directly determined, such settling could potentially lead to a small mass-dependent fractionation favoring retention of heavy isotopes deeper in the Sun, i.e., the same sense of fractionation as that caused by inefficient Coulomb drag in the SW (34). Other changes to the original isotopic composition of heavy elements in the photosphere are unlikely given that there is no mixing of nuclear processed matter into the convective zone (11). Fractionation mechanisms hypothesized as potentially operating in the solar atmosphere, e.g., mass-independent effects induced during dissociation of CO molecules in a cool layer of the chromosphere (35), are unlikely to lead to quantitative changes of the photospheric (or SW) oxygen isotopic abundances given the high temperatures ($>3000\text{ K}$) involved and rapidity of isotope exchange back-reactions and reservoir mixing in this dynamic environment. Thus, the large oxygen isotopic differences observed between the Sun and meteoritic or terrestrial samples are not caused by changes in solar matter, but instead reflect processes acting to induce mass-independent shifts in the oxygen isotopic compositions of planetary materials.

Implications for the solar nebula. The only known materials with oxygen isotopic compositions close to those of the Sun are the CAIs and other refractory phases of chondritic meteorites (Fig. 4), interplanetary dust (36), and at least one comet (37). CAIs are thought to be the earliest solar system condensates, and most crystallized with spallogenic beryllium and lithium, probably from proton bombardment in the magnetically

active environment near the still-accreting proto-Sun (38, 39). That their oxygen isotopes are dominated by a solar, rather than planetary, component reinforces their status as xenoliths (2, 40, 41) in the asteroid belt. However, most CAIs, including ultrarefractory hibonite grains (Fig. 4), are not quite as ^{16}O -enriched as the Sun, implying some mixing with isotopically heavier oxygen from other solar system reservoirs.

Our results suggest that essentially all planetary objects in the inner solar system ($<5\text{ AU}$) have oxygen isotopic compositions distinct from the average of the solar nebula from which they formed, having been enriched by $\sim 70\text{‰}$ in both $^{18}\text{O}/^{16}\text{O}$ and $^{17}\text{O}/^{16}\text{O}$ by one or more non-mass-dependent fractionation processes before accretion. Considering that oxygen is by far the most abundant element in the terrestrial planets, this points to efficient, planetary-scale processes that, if based on molecular speciation, must involve the dominant O-bearing molecules in the solar nebula: CO, H_2O , and/or silicate dust (SiO , MgO , FeO , and others in combination). A leading hypothesis, which predicted our results (6), invokes isotope-selective self-shielding during ultraviolet (UV) photolysis of CO. Because of their relatively low abundances, the C^{17}O and C^{18}O isotopomers continue to be dissociated after all the photons capable of dissociating C^{16}O have been absorbed; the liberated ^{17}O and ^{18}O atoms are then rapidly sequestered into H_2O ice and eventually are incorporated into oxide and silicate grains (7, 10). The places and times where this results in a slope 1 fractionation trajectory on the oxygen three-isotope plot (i.e., in pure enrichment or depletion of ^{16}O) are constrained by gas column

Fig. 4. Oxygen three-isotope plot showing representative compositions of major primary components of solar system matter, the solar wind (SW), and our preferred value for the Sun. All data fall predominantly on a single mixing line characterized by excesses (lower left) or depletions (upper right) of ^{16}O relative to all samples of the Earth and Moon. Plotted are the most ^{16}O -enriched solar system samples: an unusual chondrule (47); individual platy hibonite grains (55), which are ultrarefractory oxides from carbonaceous chondrites (CC); water inferred to have oxidized metal to magnetite (56) in ordinary chondrites (OC); very ^{16}O -depleted water from the CC Acfer 094 (3), and whole CAIs from CC (19); and chondrules from CC and OC (19), bulk Earth (mantle), and Mars (SNC meteorites). The mass-dependent fractionation trajectory of primary minerals in FUN inclusions and the pure ^{16}O (slope 1.0) line (57) are also shown.



density and UV flux. If isotopic self-shielding is the mechanism, the distribution of isotopically heavy water ice and its interaction with the rock reservoir (42, 43) are critical, but presently poorly understood, problems.

Measurements of low-density anhydrous interplanetary dust particles (IDPs), which if derived from comets (as hypothesized) should not have exchanged oxygen isotopes with ices, do not support a dominantly ^{16}O -enriched dust reservoir as input into the solar nebula (44); however, the cometary origin of these IDPs is not firmly established, and many of the materials of the Jupiter family Comet Wild 2 sampled by the Stardust Mission originated in the inner solar system (37). Models that place CO gas and H_2O ice oxygen isotope fractionation in the cold molecular cloud preceding formation of the solar nebula [e.g., (10, 45)] usually assume that average primordial dust is relatively ^{16}O -rich, although this is not strictly required. That such a component has not been found in any appreciable abundance in meteorites or IDPs has led to an alternative interpretation: formation of planetary materials by mixing relatively $^{17,18}\text{O}$ -enriched ($\Delta^{17} \approx 0$) dust with (^{16}O -rich) solar gas at much greater than average solar nebula proportions of dust to gas (27). This mixing scenario relegates isotopic self-shielding to a minor effect and has the benefit of alleviating some uncomfortably tight chronological constraints on oxygen isotope processing. By contrast, the most ^{16}O -depleted solar system material found in meteorites (inset, Fig. 4) is thought to have been produced via direct oxidation by ^{16}O -depleted water ice (3), in line with expectations based on the isotopic-abundance-driven self-shielding mechanism. Additionally, astronomical observations of oxygen isotopic abundances in CO gas in accretion disks surrounding young stellar objects (46) have yielded evidence for non-mass-dependent fractionation consistent with the self-shielding UV photolysis model.

One chondrule has been found that is even more ^{16}O -enriched (47) than the solar value measured here; the significance of this is not clear. In a self-shielding model, it is possible that the chondrule acquired its oxygen through interaction with a CO-rich gas that had already been depleted of heavy isotopes of oxygen via UV-photolysis of the rare isotopologs. This would require transport or sequestering of the $^{17,18}\text{O}$ out of the region in which this chondrule or its precursors formed. In the mixing model (27), the chondrule would have formed in a strongly dust-depleted region of the solar nebula.

Alternative hypotheses to isotope-selective photochemical self-shielding are based on laboratory experiments (48) and observations (49) of gas-phase (9) or surface (8) chemistry wherein non-mass-dependent isotopic effects are manifestations of molecular symmetry-induced degeneracy effects on isotope reaction rates. So-called non-RRKM effects may also play a role during growth of silicate condensates via surface reac-

tions (8, 50); however, definitive experimental evidence for such effects on oxygen isotope abundances is currently lacking.

Analyses of $^{15}\text{N}/^{14}\text{N}$ in the same Genesis sample measured here demonstrate that planetary nitrogen is highly depleted in the light isotope ^{14}N relative to solar values to an extent not compatible with normal thermodynamically driven mass-dependent fractionation (51), possibly also pointing to a dominant role of photochemistry in setting volatile element isotope patterns in the solar accretion disk.

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- At any one time, the SW is a constant-velocity plasma; thus, isotopes vary in kinetic energy. The difference in implantation depth in the Concentrator target is ~ 1.5 nm per mass unit at the peak of the profile.
- The ~ 6.5 kV potential of the interior of the Concentrator resulted in higher implantation energies in the range of 46 to 100 keV for oxygen's dominant charge states of +6 and +7 and incident velocities of 300 to 800 km/s. Typical incident angles at the target are 10° to 55° from normal for the main portion of the target, resulting in depth profiles with peaks near 80 nm.
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Supporting Online Material

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A ^{15}N -Poor Isotopic Composition for the Solar System As Shown by Genesis Solar Wind Samples

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The Genesis mission sampled solar wind ions to document the elemental and isotopic compositions of the Sun and, by inference, of the protosolar nebula. Nitrogen was a key target element because the extent and origin of its isotopic variations in solar system materials remain unknown. Isotopic analysis of a Genesis Solar Wind Concentrator target material shows that implanted solar wind nitrogen has a $^{15}\text{N}/^{14}\text{N}$ ratio of $2.18 \pm 0.02 \times 10^{-3}$ (that is, $\approx 40\%$ poorer in ^{15}N relative to terrestrial atmosphere). The $^{15}\text{N}/^{14}\text{N}$ ratio of the protosolar nebula was $2.27 \pm 0.03 \times 10^{-3}$, which is the lowest $^{15}\text{N}/^{14}\text{N}$ ratio known for solar system objects. This result demonstrates the extreme nitrogen isotopic heterogeneity of the nascent solar system and accounts for the ^{15}N -depleted components observed in solar system reservoirs.

Matter in the solar system is, in general, very well homogenized with respect to its isotopes, which is probably a result of efficient stirring in the highly turbulent nascent solar system. However, some of the light elements—those that were mostly present in the gaseous form, such as hydrogen (H_2), nitrogen (N_2), and oxygen (CO , H_2O)—present large, sometimes extreme, variations of their isotopic ratios (that is, D/H , $^{15}\text{N}/^{14}\text{N}$, and $^{17,18}\text{O}/^{16}\text{O}$, respectively) among different solar system objects and reservoirs (1–3). The cause of such variations (whether resulting from processes internal to the solar system or inherited from a presolar history) remains unclear, in part because the initial isotopic compositions of these elements in the protosolar gas are not known. To unravel this problem, the Genesis mission sampled solar wind (SW) ions in various target materials during 27 months at Lagrangian point L1 (4). SW ions give access to the composition of the Sun and, therefore, of the protosolar nebula (PSN) (5).

Nitrogen, the fifth most abundant element in the Sun, is particularly intriguing because its isotopic composition shows variations up to a factor of ≈ 6 in solar system objects (excluding presolar grains), either at a microscopic scale or at the planetary scale (2, 6–9). Over the past few decades, numerous attempts have been made to determine the N isotopic composition of the Sun through the analysis of solar ions implanted in lunar soils; however, both ^{15}N -rich and ^{15}N -poor components were found on minerals from lunar soils (8, 9). These variations were first thought to represent a secular change of the nitrogen isotopic composition of the SW (9), but no known solar process could be invoked to produce these

variations, and mixing between solar and planetary N was later advocated (7, 10). More recently, ion-probe depth profiling of single lunar grains demonstrated that at a depth of ≈ 50 nm, solar H (D-free) was associated with ^{15}N -poor N (e.g., depleted by at least $\approx 24\%$ relative to terrestrial) and, thus, presumably of solar origin (6).

Ion-probe analysis of Genesis Concentrator silicon carbide target. We report here the precise isotopic analysis of SW N collected by implantation into a silicon carbide (SiC) quadrant of the target of the Genesis Solar Wind Concentrator, an electrostatic mirror (11) that increased the fluence of some SW elements by a factor up to ~ 50 (12, 13). We analyzed along a traverse of the quadrant by secondary ion mass spectrometry using the Cameca 1280HR2 instrument recently installed at CRPG Nancy, France (14). This instrument has improved transfer optics and stability of primary and secondary beams that permits very-high-mass (M) resolution (up to $M/\Delta M \approx 30,000$) and high-precision isotope ratio measurements in multicollection mode. The SiC quadrant was sputtered using a 10-kV Cs^+ primary beam, and the resulting secondary $^{12}\text{C}^{14}\text{N}^-$ and $^{12}\text{C}^{15}\text{N}^-$ ions were accelerated at -10 kV (total impact energy of 20 kV) and analyzed in multicollection electron multiplier mode at a mass resolution of ≈ 8000 , so that all potential isobaric interferences were resolved (14). $^{29}\text{Si}^-$ ions were counted simultaneously to monitor the stability of the secondary beam with time (in a given depth profile), as well as to provide a way to normalize N isotope counts between different runs. Before analysis, four areas of 100 by 100 μm were cleaned of surface contamination using a low-energy ion beam (14). In each of the four cleaned areas, 6 to 10 measurements were taken by depth profiling at different locations ($\approx 10\text{-}\mu\text{m}$ diameter each). Procedural blanks were estimated by analyzing for N isotopes flight-spares SiC target material (kept on Earth) using the same analytical conditions and were found to be negligible ($<1\%$) for most analyses (Fig. 1). The implantation depths and fluences of SW nitrogen ions were estimated from the analysis of SiC implanted with ^{15}N ions at known

energy and fluence (14). Ion-probe instrumental mass discrimination was determined to be -29.5 ± 8.6 per mil (‰) by analyzing an SiC standard under the same conditions and comparing those data with the results previously obtained by laser ablation–static mass spectrometry (14).

Both ^{14}N and ^{15}N data define simple bell-shaped distributions as a function of depth that peak ~ 80 nm below the target's surface (Fig. 1), as expected for SW implantation at energies imposed by SW ion velocities and the Concentrator's ion acceleration (14). The measured $^{15}\text{N}/^{14}\text{N}$ ratio must be corrected for the instrumental mass fractionation (IMF) of the Genesis Concentrator, which varies as a function of position along its radius. This IMF was calibrated previously for the same SiC quadrant as a function of distance from the Concentrator's center using Ne (that is, by observing the deviation of the Ne isotopic composition extracted by laser ablation from known SW compositions) (15). From charge/mass considerations, the fractionation per mass unit of N (and O) isotopes should be comparable to that of Ne isotopes (11, 15); this relation allows the $^{15}\text{N}/^{14}\text{N}$ ratio to be corrected for the Concentrator's IMF on the basis of the Ne isotope fractionation. The Ne isotopic ratio measured in SiC at a distance of 18 to 20 mm from the Concentrator's center is not fractionated relative to the bulk SW neon isotopic composition (15, 16). Thus, we assume safely that the N isotopic composition of areas at 19-mm distance is that of the nonfractionated SW nitrogen. For other areas at 11 and 9 mm from the center, we use the Ne isotopic data to correct for Concentrator's isotopic fractionation (Table 1). However, these corrections are small—on the order of a few per mil (maximum: 19‰)—and mostly within stated errors. All detailed results, after corrections for the IMFs of both the Concentrator and the ion probe, are given in Table 1.

Isotopic composition of solar nitrogen. Our measurements yield a $^{15}\text{N}/^{14}\text{N}$ ratio for the SW of $2.178 \pm 0.024 \times 10^{-3}$ (95% confidence level), corresponding to $\delta^{15}\text{N} = -407 \pm 7\text{‰}$, where

$$\delta^{15}\text{N} = \left[\frac{\left(\frac{^{15}\text{N}}{^{14}\text{N}} \right)_{\text{sample}}}{\left(\frac{^{15}\text{N}}{^{14}\text{N}} \right)_{\text{ATM}}} - 1 \right] \times 1000 \text{ and ATM refers}$$

to the isotope composition of atmospheric nitrogen ($[\frac{^{15}\text{N}}{^{14}\text{N}}]_{\text{ATM}} = 3.676 \times 10^{-3}$). By its high precision, this result definitely settles the debate on the N isotopic composition of SW (17–19): it is extremely ^{15}N -poor, as proposed in (18, 19). Such a ^{15}N -depleted component (the $^{15}\text{N}/^{14}\text{N}$ ratio not being precisely known) was previously suspected to exist in meteoritic matter (2, 20) and the jovian atmosphere (21), but it was not understood as representing the solar composition.

The Sun's bulk $^{15}\text{N}/^{14}\text{N}$ ratio can be obtained from our Genesis SW measurement after correction for isotopic fractionation taking place during acceleration of SW from the photosphere and, possibly, in the convective zone of the Sun. The effect of solar diffusive element settling plus radiative levitation (DESRL) (22) is estimated to

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result in ≈ 5 to 9‰ per atomic mass unit (amu) depletion in ^{15}N of the SW over 4.5 billion years (23). Isotopic fractionation during acceleration of the SW from the photosphere occurs for noble gases (24), but it is far less well understood or quantified when compared with elemental fractionation, which, to first order, is governed by the element's first ionization potential (FIP). The Coulomb drag model of Bodmer and Bochsler (25) considers the correlation of the isotopic fractionation with the He/H elemental fractionation, assuming that the almost-factor-of-two fractionation in these elements between photosphere and SW is due to Coulomb drag alone and not to FIP fractionation. For nitrogen isotopes, the fractionation factor would be 1.041. Independently, oxygen isotope data obtained for the same SiC quadrant (26) suggest a mass-dependent isotopic fractionation factor of 1.022 per amu. For oxygen, gravitational settling accounts for a fractionation of 1.003 over the Sun's lifetime (27) such that the effect of SW processing should be 1.019 per amu, whereas the Coulomb drag model predicts a higher fractionation factor of 1.026 per amu. We tentatively assign a SW fractionation factor of $1.041/1.026 \times 1.019 = 1.034 (\pm 0.005)$ for N isotopes, in addition to the $1.007 (\pm 0.002)$ fractionation factor due to DESRL. Thus, our estimate for the $^{15}\text{N}/^{14}\text{N}$ ratio of the bulk Sun, after correction for solar processing and propagation of all errors, is $2.268 \pm 0.028 \times 10^{-3}$ (95% confidence level; $\delta^{15}\text{N} = -383 \pm 8\text{‰}$).

This corrected $^{15}\text{N}/^{14}\text{N}$ ratio is a proxy for that of the PSN. This ratio is much lower than that of the terrestrial atmosphere (3.676×10^{-3}), but it is identical to that measured in Jupiter's atmosphere [$^{15}\text{N}/^{14}\text{N} = 2.3 \pm 0.3 \times 10^{-3}$ (21)] within the relatively large errors in the Jupiter ratio. Our solar $^{15}\text{N}/^{14}\text{N}$ is also very close to that ($2.36 \pm 0.04 \times 10^{-3}$) found in a grain of osbornite (TiN) embedded in a calcium- and aluminum-rich inclusion (CAI) from the CH/CB chondrite Isheyevo (28). The presence of TiN in a CAI is understood as reflecting high-temperature (≈ 2000 K) condensation from the PSN under reducing conditions. In detail, however, osbornite appears to be enriched in ^{15}N by $42 \pm 22\text{‰}$ relative to our PSN value. If negligible isotopic fractionation took place during high-temperature condensation, the reservoir in the accretion disk from which the osbornite condensed might have been slightly modified from the PSN composition, as, for example, in the case of oxygen isotopes in CAIs (29). The difference between our PSN value determined from present-day SW and the osbornite N isotopic composition cannot be due to contribution of nitrogen isotopes produced in the Sun over its lifetime. For instance, the $^{15}\text{N}/^{14}\text{N}$ ratio of the outer Sun could, in principle, be affected by leakage from the Sun's core in which ^{14}N is produced by the carbon-nitrogen-oxygen cycle. However, both solar physics (30) and the absence of SW He isotope variation in lunar soils exposed at different epochs (31) indicate no contribution of matter from the solar core to the convective zone. Theoretically,

nucleosynthesis of nitrogen isotopes in solar flares might also have changed the $^{15}\text{N}/^{14}\text{N}$ ratio of the outer Sun. However, the effect is predicted to be negligible in the case of spallation for N, though it might be detectable for Li (32).

Because the PSN is the most ^{15}N -depleted, as well as the most gas-rich, reservoir in the solar system, we propose that the N isotope variations among solar system bodies result from variable mixing between a ^{15}N -poor gaseous component and solids rich in nitrogen-15. Observed H, N, and O isotopic variations are consistent with variable mixtures of a PSN component with components rich in heavy and rare isotopes (D, ^{15}N , and $^{17,18}\text{O}$, respectively) (Fig. 2). Alternatively, these enrichments might have resulted from interactions between photons and matter (e.g., photochemistry, ion-molecule reactions) that took place before or during formation

of the solar system (33–36). The existence of a common origin for these strong D-, ^{15}N -, ^{17}O -, and ^{18}O -enrichments is a key question.

Planetary implications. The agreement of the Sun's and Jupiter's outer atmosphere $^{15}\text{N}/^{14}\text{N}$ is of considerable importance, because Jupiter's atmosphere is enriched in N/H (along with Ar, Kr, Xe, C, and S) by about a factor of 3 compared with the solar photospheric elemental ratios (37, 38). We assume that the N isotopic composition of Jupiter's atmosphere is representative of the whole planet. These enhancements are usually interpreted as indicating that Jupiter is a mixture of solar nebula gas (the source of H and He) and outer solar system planetesimals [the source of the other, less volatile, elements (37)]. If this interpretation is correct, then only about one-fourth of the N in Jupiter is of nebula origin. Nevertheless, Jupiter has preserved

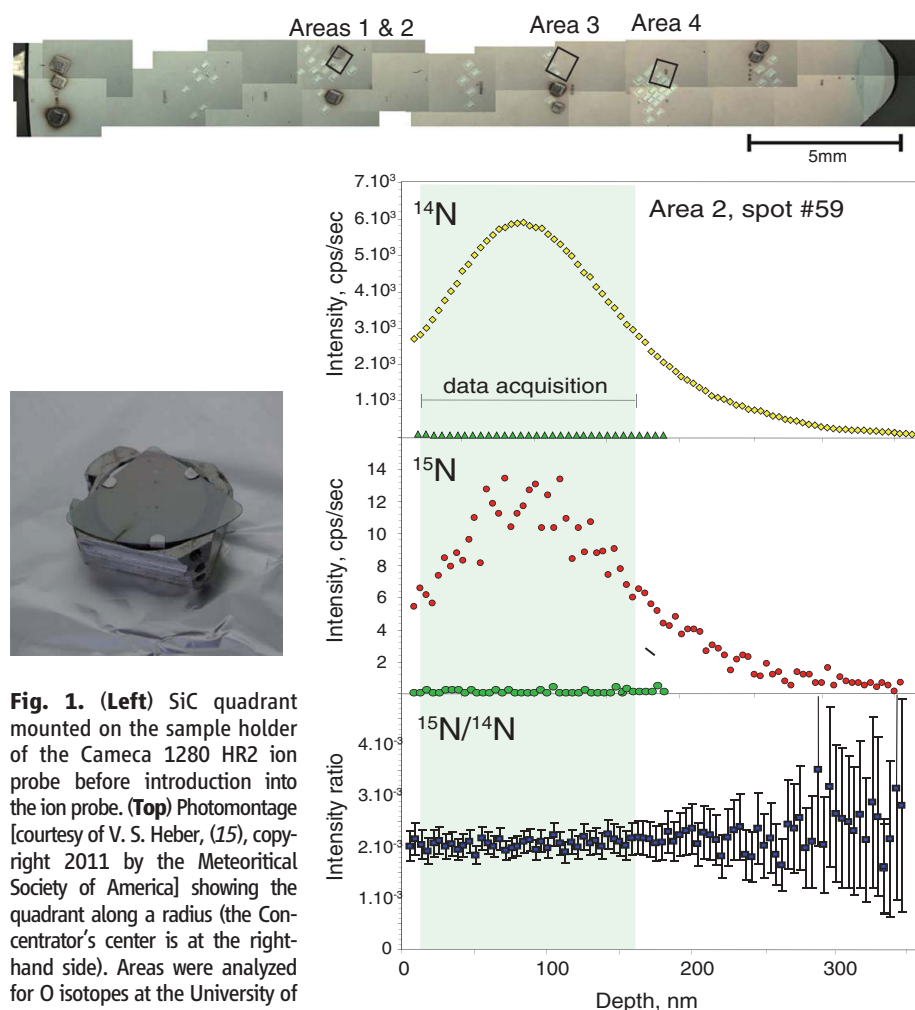


Fig. 1. (Left) SiC quadrant mounted on the sample holder of the Cameca 1280 HR2 ion probe before introduction into the ion probe. (Top) Photomontage [courtesy of V. S. Heber, (15), copyright 2011 by the Meteoritical Society of America] showing the quadrant along a radius (the Concentrator's center is at the right-hand side). Areas were analyzed for O isotopes at the University of California Los Angeles, Ne and Ar isotopes at ETH Zurich, and N isotopes at CRPG Nancy (areas delineated by squares are those analyzed for N). (Right) Example of evolution of the ^{14}N (analyzed as $^{12}\text{C}^{14}\text{N}$) and ^{15}N (analyzed as $^{12}\text{C}^{15}\text{N}$) intensities and of the $^{15}\text{N}/^{14}\text{N}$ intensity ratios as function of depth inside the SiC target (the target surface is at the left-hand side of the diagram). Green symbols (triangles and circles) represent data obtained for a blank SiC target identical to the flight target. The light green shaded area delineates data used for quantification of the SW N composition. These data were selected by removing the first five data points closest to the surface and by selecting data defining a Gaussian curve relative to the top of the bell-shaped distribution. Yellow diamonds, ^{14}N as a function of depth; red circles, ^{15}N as a function of depth; blue squares, $^{15}\text{N}/^{14}\text{N}$ ratios. cps, counts per second. Error bars were computed from counting statistics.

the solar $^{15}\text{N}/^{14}\text{N}$ ratio, requiring that the N in the planetesimal contribution had a low $^{15}\text{N}/^{14}\text{N}$ ratio, with only very small contributions from the very high $^{15}\text{N}/^{14}\text{N}$ ratio observed in cometary HCN and CN (39) or even the more modest $^{15}\text{N}/^{14}\text{N}$ ratio enrichments measured in bulk inner solar system carbonaceous chondrite material. Thus, either the model for the origin of jovian volatiles is not correct, or the cometary HCN-CN $^{15}\text{N}/^{14}\text{N}$ may not be representative of outer solar system, possibly even cometary, matter.

The extremely ^{15}N -poor composition for the PSN provides the ^{15}N -depleted component required to account for the N isotope variations previously recognized in solar system objects (Fig. 2). Presolar phases, which show a range of $^{15}\text{N}/^{14}\text{N}$ ratios from ~ 50 to $\sim 20,000$ (40), are usually considered to be negligible to the solar system N budget. The average abundances in chondrites of presolar diamonds, SiC, and graphite are ~ 1000 , ~ 10 and ~ 1 parts per million (ppm), respectively (40), and their N contents (with the exception of Si_3N_4) range from ~ 500 to $\sim 10,000$ ppm (41). Contrary to unequivocal presolar grains, the origin of meteoritic nanodiamonds is unclear because, although they can contain presolar xenon (42), their C isotopic composition is solar (43). The nanodiamond $^{15}\text{N}/^{14}\text{N}$ ratio [$2.40 \pm 0.03 \times$

10^{-3} (43)] is similar to that of the PSN, which might also point to a solar origin. It is possible that most nanodiamonds formed in the solar system, with only a small fraction (those hosting isotopically anomalous Xe) being inherited from other stellar systems. In detail, nanodiamonds, which comprise several populations, are enriched in ^{15}N by $58 \pm 17\%$ relative to the PSN composition. Hence, they might have sampled regions of the disk enriched in ^{15}N by addition of a minor, presumably nucleosynthetic, component, possibly the one hosting the anomalous Xe.

Our result also has implications for the origin of volatile elements in terrestrial planets. These elements have similar N and H isotopic compositions to those of the sources of some chondritic materials (Fig. 2). Mixing between a D-rich, ^{15}N -rich component, as observed in some comets with a solar reservoir (Fig. 2), cannot account for the relative homogeneity (ignoring factors of 1.5 or less) of N and H isotopic ratios of inner planets and meteorites. Instead, this homogeneity suggests relatively efficient stirring and mixing of at least three (N, H) components in the inner solar system.

Nitrogen isotopic variations in meteorites provide a new cosmochemical tracer for understanding chemical and thermodynamical heterogeneities during condensation, dust aggregation

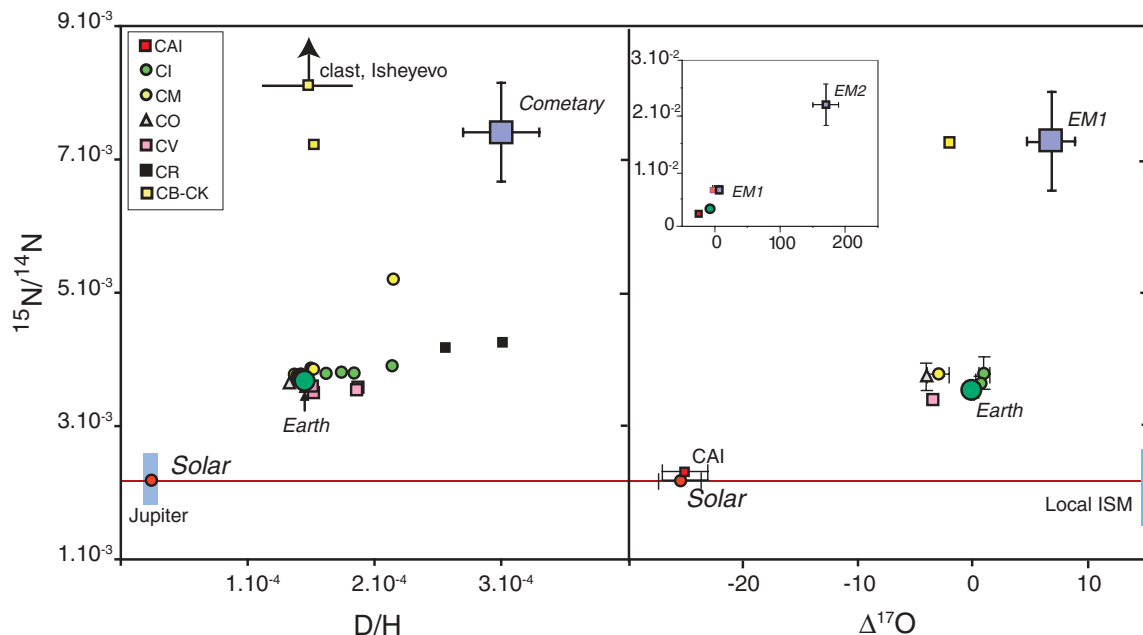
and coalescence, and parent-body processing. Variations of the bulk N isotopic compositions of meteorites (44–46) are now consistent with mixing between a ^{15}N -rich end-member, hosted by organics and dominating the carbonaceous chondrite inventory, and a ^{15}N -depleted component derived from the PSN, preferentially hosted by silicate and metal phases. Insoluble organic matter in meteorites can be extremely rich in ^{15}N on a micron scale (47, 48) and was probably the source of the large ^{15}N enrichments observed in CR/CB meteorites (49, 50). The incorporation of PSN nitrogen could have taken place either during nebular condensation or by solution in molten silicates and could have been most efficient under reducing conditions imposed by the composition of the PSN. From the composition of the PSN and adopting canonical conditions for the inner solar system [pressure $P = 10^{-3}$ atm, temperature $T = 1500$ K, $\log f_{\text{O}_2} \approx -19$ (where f_{O_2} is the oxygen fugacity) (51)], ~ 4 to 100 ppm of solar N could have been dissolved from the PSN into a basaltic melt [the only composition for which the N solubility as a function of f_{O_2} has been measured (52)], making a substantial fraction of meteoritic nitrogen (range: 1 to 1000 ppm N). For the same P - T conditions and at $\log f_{\text{O}_2} \approx -20.3$ [for enstatite chondrite

Table 1. Results from the analysis of different areas along the Concentrator's SiC quadrant (SOM, figs. S5 to S8). (Top) For each area, 6 to 10 spots were analyzed, and 60 counting runs ($^{12}\text{C}^{14}\text{N}$, $^{12}\text{C}^{15}\text{N}$, and ^{30}Si) were made for each spot. Each spot value given here represents the average of the corresponding runs. The 95% confidence intervals (CIs) for each spot are taken as the $2\sigma/(n)$ values (where n is the number of data points in the spot). The average values for each area have been weighted by the 95% CIs of each spot. The N isotopic ratios can also be obtained by computing the ratios of the background-corrected sums of ^{15}N and ^{14}N counts, which gives

the same values as above within 2 to 3‰, well within errors. (Bottom) The averages of each area have been corrected for the Concentrator's fractionation using the Ne isotopic ratios for the corresponding areas [Ne data from (15)], assuming similar isotopic fractionation per mass unit for Ne and N [see (13, 15) for justification]. The weighted average of all areas' data are given at the bottom right of the lower section of the table. These values are finally corrected for the instrumental mass fractionation in the ion probe (corr. IMF), determined from the analysis of standard SiC. ATM refers to the nitrogen isotopic composition of the terrestrial atmosphere.

Area 1			Area 2			Area 3			Area 4		
Spot #	$^{15}\text{N}/^{14}\text{N}$ measured	95% CI	Spot #	$^{15}\text{N}/^{14}\text{N}$ measured	95% CI	Spot #	$^{15}\text{N}/^{14}\text{N}$ measured	95% CI	Spot #	$^{15}\text{N}/^{14}\text{N}$ measured	95% CI
4	2.233×10^{-3}	2.40×10^{-4}	59	2.108×10^{-3}	2.88×10^{-5}	69	2.110×10^{-3}	1.06×10^{-4}	81	2.150×10^{-3}	7.12×10^{-5}
5	2.180×10^{-3}	2.27×10^{-4}	60	2.117×10^{-3}	8.25×10^{-5}	70	2.111×10^{-3}	9.57×10^{-5}	82	2.220×10^{-3}	1.19×10^{-4}
6	2.093×10^{-3}	2.45×10^{-4}	61	2.161×10^{-3}	6.50×10^{-5}	71	2.114×10^{-3}	1.07×10^{-4}	83	2.211×10^{-3}	1.12×10^{-4}
7	2.168×10^{-3}	3.17×10^{-4}	62	2.123×10^{-3}	9.21×10^{-5}	73	2.080×10^{-3}	9.76×10^{-5}	84	2.145×10^{-3}	1.01×10^{-4}
8	2.009×10^{-3}	2.52×10^{-4}	63	2.082×10^{-3}	9.14×10^{-5}	74	2.195×10^{-3}	1.24×10^{-4}	85	2.111×10^{-3}	9.55×10^{-5}
10	2.195×10^{-3}	2.61×10^{-4}	64	2.175×10^{-3}	9.50×10^{-5}	75	2.050×10^{-3}	1.11×10^{-4}	86	2.151×10^{-3}	9.09×10^{-5}
11	2.129×10^{-3}	2.05×10^{-4}	65	2.104×10^{-3}	1.05×10^{-4}	76	2.189×10^{-3}	8.27×10^{-5}			
			66	2.073×10^{-3}	1.07×10^{-4}	77	2.132×10^{-3}	1.01×10^{-4}			
			67	2.100×10^{-3}	7.52×10^{-5}	78	2.108×10^{-3}	9.41×10^{-5}			
			68	2.171×10^{-3}	1.09×10^{-4}	80	2.100×10^{-3}	1.12×10^{-4}			
Average	2.143×10^{-3}	9.00×10^{-5}		2.116×10^{-3}	2.00×10^{-5}		2.121×10^{-3}	3.10×10^{-5}		2.158×10^{-3}	3.80×10^{-5}
Dist./Concentrator's center (mm)		$^{20}\text{Ne}/^{22}\text{Ne}$ measured	95% CI	$^{15}\text{N}/^{14}\text{N}$ measured	95% CI	$(^{15}\text{N}/^{14}\text{N})$ corr. Concentrator's fractionation		95% CI			
Area 1	19	13.78	0.04	2.143×10^{-3}	9.00×10^{-5}	2.143×10^{-3}		9.01×10^{-5}			
Area 2	19	13.78	0.04	2.116×10^{-3}	2.00×10^{-5}	2.126×10^{-3}		2.02×10^{-5}			
Area 3	11	13.48	0.08	2.121×10^{-3}	3.10×10^{-5}	2.098×10^{-3}		3.13×10^{-5}			
Area 4	7	13.26	0.06	2.158×10^{-3}	3.80×10^{-5}	2.117×10^{-3}		3.76×10^{-5}			
Average						2.114×10^{-3}		1.5×10^{-5}			
corr. IMF						2.178×10^{-3}		2.4×10^{-5}			
$\delta^{15}\text{N}/\text{ATM} (\text{‰})$						−407		7			

Fig. 2. Variations of the nitrogen isotopic composition as a function of the D/H ratio (**left**), the oxygen isotopic composition (**right**), expressed as $\Delta^{17}\text{O}$, which is the distance from the mass-dependent terrestrial fractionation line equal to $0.52 \times \delta^{18}\text{O}$ [1]. For the nitrogen versus oxygen isotopic compositions, two end members are considered: End-member 1 (EM1) is the cometary composition for N (39) and the isotope composition estimated for the aqueous component that altered carbonaceous chondrites (29); end-member 2 (EM2) is defined with the N isotope composition of the most ^{15}N -rich component found in the Isheyevo CH/CB chondrite (47) and with the matrix of the carbonaceous chondrite Acfer 094 which has the most extreme O isotope composition found in meteorites and is thought to be a remnant of early solar system water (53). (Inset) Same diagram with an expanded scale.



composition (51)], ~230 ppm solar N would have been dissolved, which, by mixing with ~1000-ppm organic N with $\delta^{15}\text{N} \approx +40\%$ typical of nitrogen in CI/CM carbonaceous chondrites (46), will yield a $\delta^{15}\text{N}$ value of $\sim -40\%$, as observed in enstatite meteorites (46). Thus, the isotopes of nitrogen also have a great potential for understanding chondrite evolutionary processes (e.g., chondrule formation events) and the evolution of planetesimals in their early stage when they were partially molten.

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Carbon-Based Supercapacitors Produced by Activation of Graphene

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Supercapacitors, also called ultracapacitors or electrochemical capacitors, store electrical charge on high-surface-area conducting materials. Their widespread use is limited by their low energy storage density and relatively high effective series resistance. Using chemical activation of exfoliated graphite oxide, we synthesized a porous carbon with a Brunauer-Emmett-Teller surface area of up to 3100 square meters per gram, a high electrical conductivity, and a low oxygen and hydrogen content. This sp^2 -bonded carbon has a continuous three-dimensional network of highly curved, atom-thick walls that form primarily 0.6- to 5-nanometer-width pores. Two-electrode supercapacitor cells constructed with this carbon yielded high values of gravimetric capacitance and energy density with organic and ionic liquid electrolytes. The processes used to make this carbon are readily scalable to industrial levels.

Supercapacitors store energy by forming a double layer of electrolyte ions on the surface of conductive electrodes. Supercapacitors are not limited by the electrochemical charge transfer kinetics of batteries and thus can operate at very high charge and discharge rates and can have lifetimes of over a million cycles (1). However, the energy stored in supercapacitors is currently an order of magnitude lower than that of batteries, which limits their adoption to those applications that require high cycle life and power density. The energy density of existing state-of-the-art supercapacitor devices, which are mainly based on porous activated carbon (AC), is about 4 to 5 watt-hour (Wh)/kg, whereas that of lead acid batteries is in the range of 26 to 34 Wh/kg (2). A typical AC material, with a Brunauer-Emmett-Teller (BET) specific surface area (SSA) in the range of 1000 to 2000 m^2/g and a pore size distribution in the range of 2 to 5 nm, has a gravimetric capacitance of 100 to 120 F/g in organic electrolytes (3). Research has thus been focused on increasing energy density without sacrificing cycle life or high power density (4). An increased capacitance in the organic electrolyte tetraethylammonium tetrafluoroborate (TEA BF_4) in acetonitrile (AN) by using carbide-derived carbons (CDCs) has been reported (5). Metal oxides such as RuO_2 or MnO_2 (6), MoO_3 (7), and electronically conducting polymers (8), or their composites, have been used to increase specific

capacitance via pseudo-capacitive redox reactions. Although capacitances of up to 1300 F/g (such as with MnO_2) have been reported in aqueous electrolytes (9), the low electrical conductance, poor

compatibility with organic electrolytes, and short cycle life have limited the practical application of these pseudo-capacitive materials. Carbon nanotubes (CNTs), especially single-walled CNTs (SWNTs), have an ideal limit SSA of 1300 m^2/g (10), can have high electrical conductance along the tubes, and demonstrate good performance in organic electrolyte (11). However, the high cost for mass production of high-quality SWNTs is a challenge for the commercialization of SWNT-based supercapacitors.

Graphene has a theoretical SSA of 2630 m^2/g and a very high intrinsic electrical conductivity in plane as well as high mechanical strength and chemical stability (12). Graphene-based material derived from graphite oxide (GO) is now being manufactured in ton quantities at low cost (13). We have previously demonstrated supercapacitors that were based on reduced graphene oxide with capacitance values of approximately 130 and 100 F/g in aqueous KOH and organic electrolytes, respectively (14). With a low equivalent series resistance (ESR), the supercapacitor performance did not show much degradation with an increase in the scan rate. Various graphene-based materials derived from GO have reported

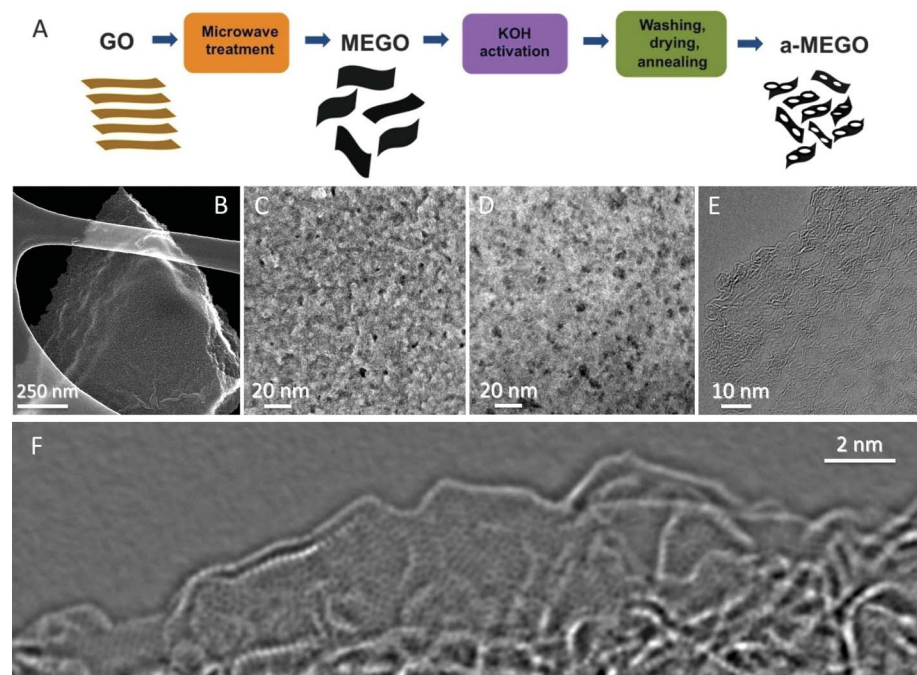


Fig. 1. (A) Schematic showing the microwave exfoliation/reduction of GO and the following chemical activation of MEGO with KOH that creates pores while retaining high electrical conductivity. (B) Low-magnification SEM image of a 3D a-MEGO piece. (C) High-resolution SEM image of a different sample region that demonstrates the porous morphology. (D) ADF-STEM image of the same area as (C), acquired simultaneously. As seen, a-MEGO contains micro- and mesopores with a distribution of sizes between ~1 and ~10 nm. (E) High-resolution phase contrast electron micrograph of the thin edge of an a-MEGO chunk, taken at 80 kV. There is a variation in focus across the image because of the sloped nature of the sample and changes in sample thickness. The image shows the presence of a dense network of nanometer-scale pores surrounded by highly curved, predominantly single-layer carbon. (F) Exit wave reconstructed HR-TEM image from the edge of a-MEGO. The in-plane carbon atoms are clearly resolved, and a variety of n -membered carbon rings can be seen. Substantial curvature of the single-carbon sheets is visible, with the in-plane crystallinity being preserved.

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high-end capacitance values of ~ 200 F/g in aqueous electrolytes (15, 16), ~ 120 F/g in organic electrolytes (16, 17), and ~ 75 F/g in an ionic liquid (18). Recently, supercapacitors using oriented graphene grown on nickel by means of chemical vapor deposition were reported (19) that demonstrated efficient filtering of 120 Hz current with a resistance capacitance (RC) time constant of less than 0.2 ms, but at the cost of effective energy storage because of the very low density of the electrode material.

To date, the reported SSA values of carbon materials derived from GO have been well below 2630 m^2/g . Here, we report a simple activation with KOH of microwave exfoliated GO (MEGO) and thermally exfoliated GO (TEGO) to achieve SSA values up to 3100 m^2/g . As described in (20), we prepared MEGO powders by irradiating GO in a microwave oven. The as-made MEGO powder was then placed in KOH solution, followed by filtration and drying, to form a series of MEGO/KOH mixtures for chemical activation. Each MEGO/KOH mixture was put in a tube furnace under flowing argon at a pressure of about 400 torr and heated at 800°C for 1 hour. A schematic of this activation process is shown in Fig. 1A. Chemical activation has been extensively used to obtain porous ACs (21). KOH activation has been used on CNTs (22), carbon nanofibers (23), and polyacrylonitrile-carbon nanotube composites (24), and improved porosity and enhanced supercapacitor performance were reported. It is suggested that the activation of carbon with KOH (25) proceeds as $6\text{KOH} + \text{C} \leftrightarrow 2\text{K} + 3\text{H}_2 + 2\text{K}_2\text{CO}_3$, followed by decomposition of K_2CO_3 and/or reaction of $\text{K}/\text{K}_2\text{CO}_3/\text{CO}_2$ with carbon.

The activation with KOH generated nanoscale pores in the product carbon. The SSA of the activated MEGO (a-MEGO) could be readily controlled by the ratio of KOH versus MEGO (fig. S1). The scanning electron microscopy (SEM) image in Fig. 1B shows the morphology of a typical a-MEGO piece. Figure 1 shows high-resolution SEM (Fig. 1C), annular dark field scanning trans-

mission electron microscopy (ADF-STEM) (Fig. 1D), and high-resolution TEM (HR-TEM) (Fig. 1, E and F) images of the microstructure. These images [and additional images (fig. S2 and movie S1)] clearly indicate that the activation process etches the MEGO and has generated a three-dimensional (3D) distribution of what are referred to as meso- and micropores in the porous materials literature. The activation with KOH yields a continuous 3D network of pores of extremely small size, ranging from ~ 1 nm to ~ 10 nm. Thus, it appears that the chemical activation is not merely digesting the MEGO but also dramatically restructuring it. (The 3D nature of these very small pores makes a statistically accurate quantitative analysis with EM of the distribution of pore sizes difficult because not all pores are visible in a given image.) The spherical aberration-corrected HR-TEM image presented as Fig. 1E (taken at 80 kV so as to prevent electron beam damage) further corroborates a dense pore structure with a continuous three-dimensional network of highly curved, predominantly atom-thick walls. An exit-wave reconstructed image (Fig. 1F) taken with the Transmission Electron Aberration-Corrected Microscope (TEAM) instrument (spherical and chromatic aberration correction, at 80 kV) clearly resolves the individual carbon atoms in the structure. This image shows that a-MEGO is composed of n -membered rings in plane, with n varying between 5 and 8. Additionally, it is clear that even as the walls bend through high degrees of curvature the in-plane crystallinity is preserved.

Electron paramagnetic resonance (EPR) data (fig. S3) indicate an unpaired spin count at the parts-per-million level. This—in hand with the very low H and O content in the a-MEGO indicated by elemental analysis—shows that a-MEGO has a small fraction of edge atoms. (If a large fraction of the C were edge atoms, they would either be functionalized or would be present as “dangling bonds” that would register an EPR signal.)

Characterization of a sample of a-MEGO by means of synchrotron powder x-ray diffraction (XRD) and x-ray photoelectron spectroscopy (XPS) is shown in Fig. 2, A and B. Comparison with MEGO indicates that the (002) peak of a-MEGO has a markedly reduced intensity and is dramatically broadened. These results are consistent with the observations from HR-TEM, which indicate that a-MEGO is composed of predominantly single-carbon sheets: Thus, a strong decrease in the (002) peak would be expected. A large increase in the low-angle scatter from a-MEGO versus MEGO is also noted, which is consistent with the presence of a high density of pores. In the XPS $\text{C}1s$ spectrum of MEGO shown in Fig. 2B, the tail between 286 and 290 eV is due to C-O groups and energy loss “shake-up” features (26). These oxygen-containing groups were strongly suppressed after activation, with two new peaks appearing between 292 and 296 eV in the a-MEGO sample that were assigned as $\text{K}2p$ peaks. The $\text{K}2p$ peaks (<2 atomic % as determined from XPS) in a-MEGO are due to potassium residue, primarily as K_2CO_3 with a small amount of KOH. Quantification of the amount of sp^2 -bonding can be determined by measuring the ratio between π^* bonding and $\pi^* + \delta^*$ bonding by using electron energy loss spectroscopy (EELS) (27). A comparison of the carbon K near-edge structure for a-MEGO and graphite of equivalent thickness is presented in Fig. 2C. With the assumption that the sp^2 bonding in the graphite reference spectra is 100%, the a-MEGO was found to have 98% ($\pm 2\%$) sp^2 bonding. Complementary measurements were also made by XPS, reaching similar conclusions (further details of the fitting procedures can be found in fig. S4). Micro Raman spectroscopy (fig. S5A) and Fourier transform infrared spectroscopy (fig. S5B) are provided for completeness but did not supply additional insights about a-MEGO beyond the other methods of analysis discussed here.

State-of-the-art surface and pore-size characterization of the a-MEGO was performed by

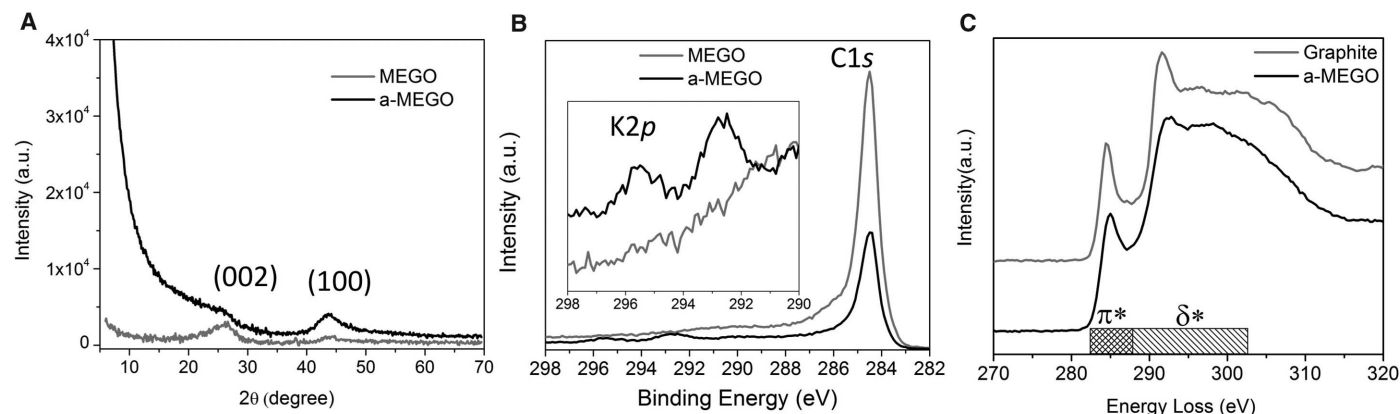


Fig. 2. Characterization of the a-MEGO material (SSA ~ 2520 m^2/g) with MEGO or graphite as a control. **(A)** Synchrotron powder XRD pattern (plotted as $\text{Cu K}\alpha$). Full width at half maximum of the (100) peak is ~ 2 degrees for both samples, indicating average in-plane crystal size of ~ 5 nm with the Scherrer equation; the

reduction of the (002) in a-MEGO indicates that there are essentially no inter-layer correlations of the carbon sheets. **(B)** XPS $\text{C}1s$ spectra, with $\text{K}2p$ region in the inset. **(C)** EELS spectra from a-MEGO and graphite. Quantification of the near-edge structure indicates that the a-MEGO has 98% ($\pm 2\%$) sp^2 bonding.

coupling high-resolution nitrogen (77.4 K) and argon (87.3 K) adsorption/desorption experiments with advanced methods based on density functional theory (DFT) (28). In addition, CO₂ adsorption at 273.2 K has been performed to assess the ultramicropores (pores of width <1 nm). These isotherms, as shown in Fig. 3A, reveal the details of the low-pressure region in which micropore filling occurs, as well as the linear plot of the argon and nitrogen isotherms that reveal pore condensation and type H2 hysteresis [according to the International Union of Pure and Applied Chemistry (IUPAC) classification] (29) for the a-MEGO sample, which is indicative of an interconnected pore system exhibiting constrictions (29, 30). For comparison, nitrogen adsorption on a MEGO control sample is also shown in fig. S6, and it is evident that the pore volume of MEGO is much smaller than that of a-MEGO; the small overall pore volume should be caused by the platelet-like structure in MEGO—likely just the gap between platelets (20). In contrast, the pores in a-MEGO have a well-defined micro-mesopore size distribution as shown in Fig. 3B, with a huge increase in pore volume (up to 2.14 cm³/g) relative to MEGO. The a-MEGO had a high nitrogen BET SSA of ~3100 m²/g (calculated in the linear relative pressure range from 0.1 to 0.3). Within this context, in a strict sense the BET method is not applicable to microporous solids, and hence the obtained BET surface area should be considered as an apparent or equivalent area only. Figure 3B displays the results of the cumulative pore volume and pore size analysis from CO₂ and nitrogen adsorption by applying a hybrid nonlocal DFT (NLDFIT) kernel, assuming a slit pore geometry for the micropores and a cylindrical-pore geometry for the mesopores, which appears (although oversimplified) to be a reasonable as-

sumption also with regard to the SEM/STEM/TEM results. The obtained pore-size/volume distribution indicates that this carbon sample is distinctive because of the existence of well-defined micro- and mesopores. The presence of ultramicropores is seen from the CO₂ data, and the analysis of the nitrogen adsorption data reveals the presence of micropores in the ~1-nm size range as well as narrow mesopores centered around 4 nm in size. The latter is in good agreement with the bimodal distribution of pore sizes observed with high-resolution EM images. A quenched solid DFT (QSDFT), which quantitatively accounts for the surface roughness (31), also has been used to obtain the pore-size distribution of a-MEGO from the nitrogen and argon adsorption data, as shown in fig. S7. The pore size distribution curves obtained from argon and nitrogen agree very well.

Using best-practice methods for determining an electrode material's performance for supercapacitors (32), we constructed and measured the performance of two-electrode symmetrical supercapacitor cells on the basis of a-MEGO (SSA ~ 2400 m²/g) and 1-butyl-3-methyl-imidazolium tetrafluoroborate (BMIM BF₄)/AN electrolyte, as shown in Fig. 4. The cyclic voltammetry (CV) testing (Fig. 4A) shows rectangular curves from 0 to 3.5 V over a wide range of voltage scan rates. The galvanostatic charge/discharge curves at three current densities are shown in Fig. 4B. The specific capacitance was calculated from the discharge curves with values of 165, 166, and 166 F/g obtained at current densities of 1.4, 2.8, and 5.7 A/g, respectively. The corresponding volumetric capacitance is ~60 F/cm³. The voltage drop at the initiation of the discharge is 0.034 V (for the current density of 1.4 A/g), suggesting a very low ESR in the test cell. A frequency response analysis (FRA) of the frequency range from 500 kHz

to 5 mHz yields the Nyquist plot shown in Fig. 4C. The plot features a vertical curve, indicating a nearly ideal capacitive behavior of the cell. From the magnified data in the high-frequency range (Fig. 4C, inset), a transition between the RC semi-circle and the migration of electrolyte was observed at a frequency of about 382 Hz, corresponding to a resistance of 2.45 ohms. The diffusion of electrolyte ions stopped at about 3 Hz, and thereafter the whole capacitance was reached (33). The voltage drop at the beginning of discharge curves was used to estimate the internal resistance. An ESR of 3.2 ohms was obtained from a-MEGO in the BMIM BF₄/AN electrolyte. Based on a series RC model, the capacitance from the FRA data as a function of frequency is shown in Fig. 4D. The capacitance decreases sharply at about 4 Hz and remains 0.035 F at 10 Hz. Supercapacitor performance of the a-MEGO (SSA ~ 3100 m²/g) in the TEA BF₄/AN electrolyte was also measured and yielded a specific capacitance of above 150 F/g as obtained from the discharge curve with a constant current of 0.8 A/g, with an ESR of 4.6 ohms (fig. S8). One other carbon (KOH-activated CDCs) using the same AN-based electrolyte with comparable specific capacitance values has been reported (34). a-MEGO has the highest gravimetric capacitance in organic electrolyte reported to date for any carbons derived from graphene-based materials.

Values for energy and power density were estimated on the basis of the supercapacitor measurements in the BMIM BF₄/AN electrolyte. Using the specific capacitance value of 166 F/g (from the discharge curve with a constant current of 5.7 A/g) and working voltage of 3.5 V, the energy density is ~70 Wh/kg for the a-MEGO in the cell. Based on a weight ratio of 30% for the active electrode material in a packaged supercapacitor device—typical

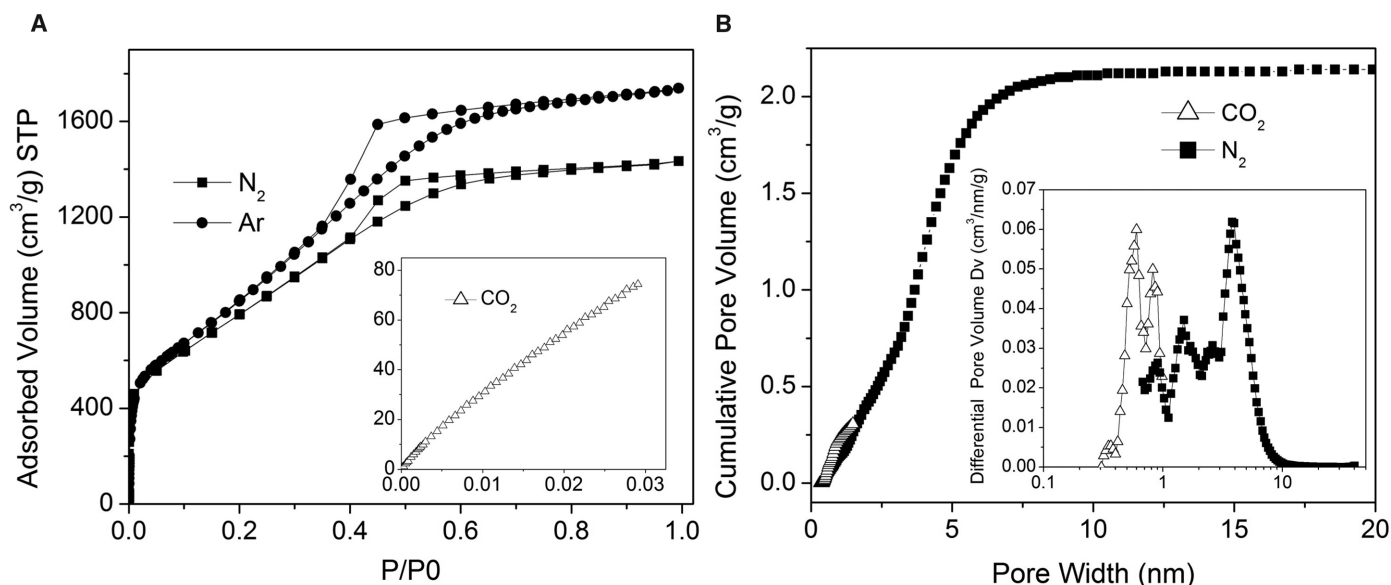


Fig. 3. Gas adsorption/desorption analysis of an a-MEGO sample (SSA ~ 3100 m²/g). **(A)** High-resolution, low-pressure N₂ (77.4 K) and Ar (87.3 K) isotherms. **(Inset)** The CO₂ (273.2 K) isotherm. **(B)** Cumulative pore

volume and **(inset)** pore-size distribution for N₂ (calculated by using a slit/cylindrical NLDFIT model) and CO₂ (calculated by using a slit pore NLDFIT model).

for large-scale AC-based supercapacitors—a practical energy density of above 20 Wh/kg for a packaged device is expected. This is four times higher than existing AC-based supercapacitors, two times higher than that reported for carbon-oxide hybrid electrochemical devices (2), and nearly equal to the energy density of lead acid batteries. At the same current density (5.7 A/g), the power density is also very high at ~ 250 kW/kg, as estimated by using the voltage drop and ESR obtained from the discharge curve. For a packaged cell, the power density of ~ 75 kW/kg is one order higher than the values from commercial carbon supercapacitors that have energy density values of 4 to 5 Wh/kg (2). This material is also very stable. After 10,000 constant current charge/discharge cycles at a current density of 2.5 A/g in neat BMIM BF₄ electrolyte (fig. S9), 97% of its capacitance was retained. We reasoned that this carbon might perform even better with smaller-diameter ions (35). With neat ethyl-methyl-

imidazolium bis(trifluoromethylsulfonyl)imide (EMIM TFSI) as electrolyte (fig. S10), the measured gravimetric capacitance of a-MEGO (SSA ~ 3100 m²/g) at 3.5 V and a current density of 0.7 A/g is 200 F/g, with an ESR of 8.6 ohms. However, the curves in fig. S10 are not as ideal as those from a-MEGO in either BMIM BF₄/AN or TEA BF₄/AN electrolyte. Other carbons (such as CDCs) using the EMIM TFSI electrolyte have been reported with comparable performance values, although the measurements were performed at an elevated temperature (60°C) (36).

The high powder conductivity of ~ 500 S/m, a C/O atomic ratio of up to ~ 35 , the very low H content, and the essential absence of dangling bonds in the a-MEGO suggest that it has a high content of sp²-bonded carbon and very few edge atoms for the samples with SSA of above 2500 m²/g. This along with the SEM, TEM, STEM, EELS, EPR, XPS, XRD, and adsorption isotherm data thus support a highly porous carbon comprised

almost entirely of single sheets of sp²-bonded carbon. This suggests that a large fraction of “negative curvature carbon” (37–39) could be present.

The excellent performance obtained for various electrolytes opens the possibility to engineer supercapacitor electrodes based on this form of carbon in order to target a wide range of applications, such as high energy, high power, or low cost. Unlike other carbon materials, no special substrates or transfer procedures are required for synthesis. For supercapacitor manufacturing, this material can be treated the same as current commercial ACs. Electrodes used for testing were of the same thickness used in commercial cells, and testing was performed using commercial collectors, separators, binders, and electrolytes. As previously stated, the processes used to synthesize this carbon electrode material are readily scalable to industrial levels. For example, we have demonstrated that this simple activation process

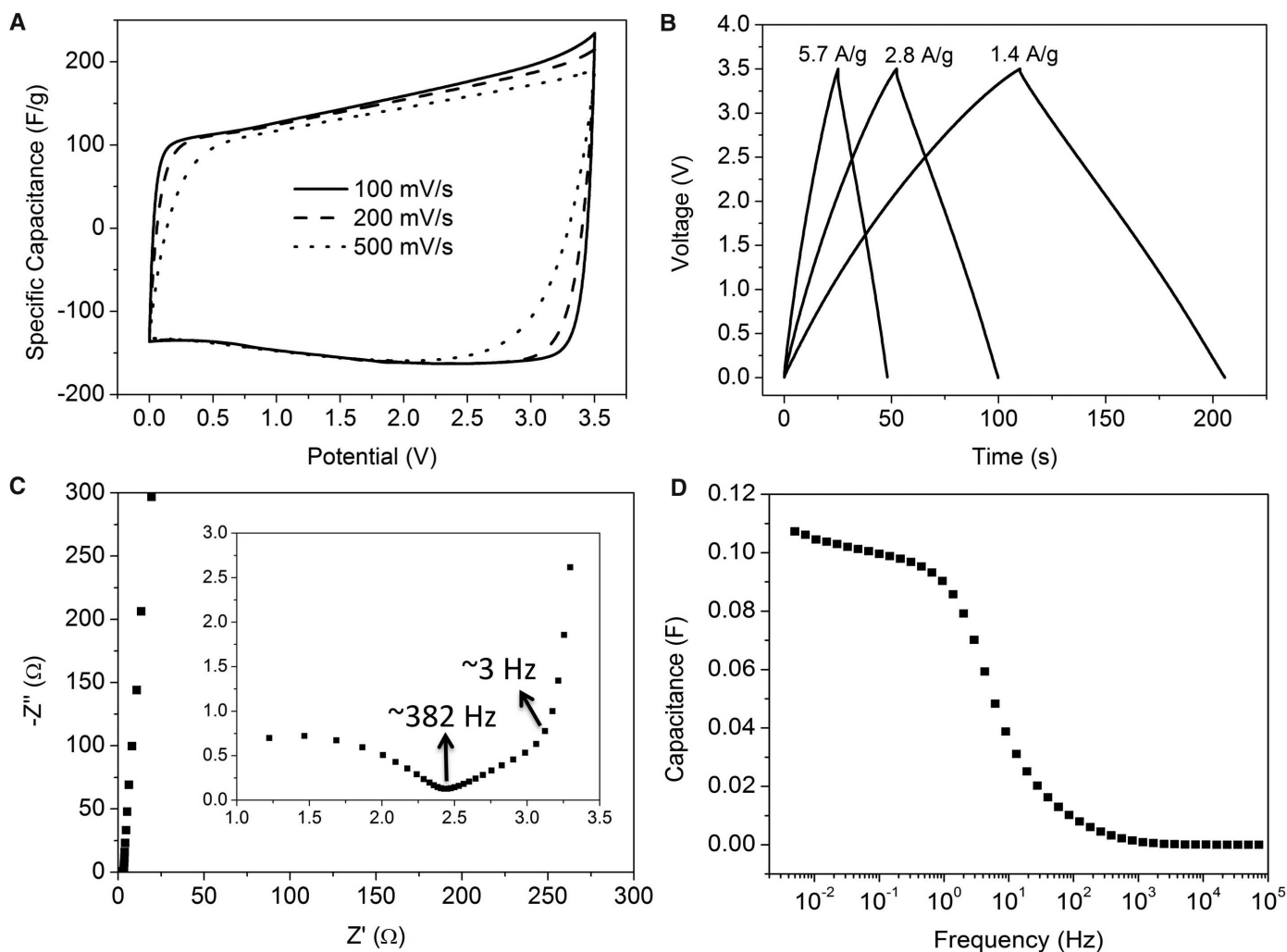


Fig. 4. Supercapacitor performance of a-MEGO (SSA ~ 2400 m²/g) in the BMIM BF₄/AN electrolyte. **(A)** CV curves for different scan rates. Rectangular shapes indicate the capacitive behavior. **(B)** Galvanostatic charge/discharge curves of a-MEGO-based supercapacitor under different constant currents. **(C)** Nyquist plot, showing the imaginary part versus the real part of im-

pedance. (Inset) The data at high-frequency ranges, with frequency values corresponding to the transition of the curves marked. **(D)** Frequency response of the gravimetric capacitance of the a-MEGO supercapacitor. Capacitances of 35 and ~ 8.8 mF remain at the frequencies of 10 and 100 Hz, respectively.

also can be applied to TEGO (figs. S11 and S12), which is already being manufactured in ton quantities (13). By use of this type of simple activation process already commercially demonstrated for ACs, scaled a-MEGO and a-TEGO production for advanced energy/power electrochemical electrical energy storage devices may be realized in a short period.

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Figs. S1 to S12

References

Movie S1

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Disorder-Enhanced Transport in Photonic Quasicrystals

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Quasicrystals are aperiodic structures with rotational symmetries forbidden to conventional periodic crystals; examples of quasicrystals can be found in aluminum alloys, polymers, and even ancient Islamic art. Here, we present direct experimental observation of disorder-enhanced wave transport in quasicrystals, which contrasts directly with the characteristic suppression of transport by disorder. Our experiments are carried out in photonic quasicrystals, where we find that increasing disorder leads to enhanced expansion of the beam propagating through the medium. By further increasing the disorder, we observe that the beam progresses through a regime of diffusive-like transport until it finally transitions to Anderson localization and the suppression of transport. We study this fundamental phenomenon and elucidate its origins by relating it to the basic properties of quasicrystalline media in the presence of disorder.

Anderson localization (1), a fundamental concept in solid-state physics, describes how introducing disorder can transform a conducting crystal into an insulator. This prediction and subsequent experiments have shown that, generally, disorder works to arrest transport in periodic systems containing disorder (2–5), as well as in fully random potentials (6–10). However, some systems still pose fundamental challenges to this concept—most notably,

quasicrystals. Quasicrystals (QCs) (11, 12) constitute an intermediate phase between fully periodic and fully disordered media: They do not have a unit cell and do not exhibit translation symmetry; nevertheless, they possess noncrystallographic rotational symmetry and long-range order and display Bragg diffraction. Although many of the properties of QCs are now well understood, some fundamental questions remain. Perhaps one of the most intriguing questions related to QCs has to do with transport. Opposite to crystals containing disorder, which exhibit Anderson localization, it has been suggested that disorder can enhance transport in QCs (13, 14). Indirect experiments have indicated that in some regimes, increasing disorder could enhance transport (14).

The electronic structure of atomic QCs has been shown to have multifractal eigenstates (15, 16), which may or may not be normalizable (thus, localized), depending on the critical exponent associated with the given state. The transport properties of QCs are directly related to the critical nature of their eigenstates, in particular, in the presence of disorder (17). QCs have been shown to exhibit counterintuitive transport properties, including extremely low conductivity that increases with both temperature (inverse Matheisen rule) and spatial disorder arising from structural defects (14). Both of these effects have been attributed (16, 18) to hopping between critical states of different spatial extents near the Fermi energy (due to inelastic electron-phonon scattering for the former and elastic scattering from structural defects for the latter). This increase in transport with disorder is directly opposite to the characteristic behavior of crystals, wherein transport is reduced with increasing disorder.

Thus far, experiments on transport in atomic QCs were carried out by the study of macroscopic conductivity. However, conductivity experiments are problematic for addressing some basic questions on QCs. First, the mechanisms proposed to explain the unusual transport in QCs assume non-interacting electrons; however, conductivity measurements inevitably incorporate electron-electron interactions. Second, conductivity measurements do not allow direct observation of wave packets, which could be a key property in unraveling the mechanisms underlying transport. With the recent progress in photonic lattices (19), manifesting analogies between light propagating in a waveguide

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array and an electron in an atomic lattice, it is natural to expect experimental studies of transport in photonic QCs. Indeed, experiments have studied the QC band structure (20), the dynamics of phasons (21), and propagation through QCs without disorder (22, 23). However, the fundamental issue of transport in photonic QCs in the presence of disorder has not previously been studied experimentally.

Here, we study photonic quasicrystals containing disorder and present the first direct experimental observation of disorder-enhanced transport in QCs by directly imaging wave packets propagating through the photonic QC containing disorder. We show that disorder considerably enhances the transport of wave packets associated with eigenstates in the proximity of a pseudogap (a sharp reduction in the density of states), the region in which the Fermi energy is found in electronic systems. Enhanced transport occurs because disorder acts to couple highly localized states near the pseudogap, and as a result, states become more extended. When disorder is further increased, we experimentally demonstrate finite-time, diffusive-like transport, as predicted for weakly disordered QCs (24). Upon increasing the disorder even further, Anderson localization prevails: the width of the wave packet shrinks, and its tails display exponential decay. Our photonic system is equivalent to a two-dimensional (2D) Penrose QC containing disorder, and the wave packet we image is analogous to the probability amplitude of an electron propagating in it; hence, our findings are relevant to conduction electrons in quasicrystal-line electronic systems.

We work with photonic lattices, in the transverse localization scheme (25), described by the paraxial equation for monochromatic light

$$i \frac{\partial \Psi}{\partial z} = \hat{H} \Psi \triangleq \left[-\frac{1}{2k} \left(\frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} \right) - \frac{k}{n_0} \Delta n(x, y) \right] \Psi \quad (1)$$

Here z is the propagation coordinate, x and y are the transverse coordinates, $\hat{H}$ is the Hamiltonian (defined by Eq. 1), Ψ is the slowly varying envelope of an optical field $E(x, y, z, t) = \text{Re}[\Psi(x, y, z)e^{i(kz - \omega t)}]$ (t is the time coordinate) of frequency ω and wave number $k = \omega n_0/c$, n_0 is the bulk refractive index, Δn is the local change in the refractive index (lattice plus disorder), and $t^2 = -1$. Equation 1 has the form of the Schrödinger equation: the equivalence emerges when $z \rightarrow t$ and $-\Delta n \rightarrow V$ (where V is the potential). Hence, the evolution of a light beam behaves like the wave packet of a quantum particle in a 2D potential, but with the coordinate z replacing time. Solving for the eigenstates $\Psi_\beta = A_\beta(x, y)\exp(-i\beta z)$ (A_β is the z -independent eigenmode), for which $\hat{H}\Psi_\beta = \beta\Psi_\beta$ (β is the energy), is the equivalent to solving for the eigenmode of a particle, where the propagation constant corresponds to the eigenenergy E . Our photonic QC containing disorder is a Penrose-tiled 2D refractive index structure on

which we superimpose 2D random disorder. The refractive index structure $\Delta n(x, y)$ corresponds to an array of parallel waveguides, ordered as a Penrose tiling, with random transverse variations whose strength is controlled at will.

Figure 1 shows the experimental scheme. We use the induction technique (26) to transform an optical intensity pattern into a z -independent refractive index structure $\Delta n(x, y, z)$, which includes both the QC lattice and the disorder (27). We study transport by launching a weak probe beam and monitoring the beam exiting the sample (28). Meaningful results are obtained by repeating experiments multiple times with many realizations of the disorder (same parameters) and ensemble-averaging over the intensity patterns at the exit face.

We now describe the results on enhanced transport in disordered QCs. The simulation results,

shown in Fig. 2A, display the ensemble-averaged width, W_{eff} (3, 29), of the beam propagating through the QC, without disorder (lower curve) and with 20% disorder (upper curve). We numerically launch a narrow Gaussian beam at a center of local 10-fold symmetry in the QC structure (center of a “flower”; see two example points marked by arrows in Fig. 2B). Without disorder, transport through the pure QC displays a “bumpy ride,” with irregular oscillations occurring because of the presence of states of very different spatial extent within small energy ranges. On the other hand, transport through the QC containing disorder is considerably enhanced (Fig. 2A, upper curve): Throughout propagation, the width of the beam propagating in the disordered QC is larger than the width of the beam propagating in the pure QC. Moving the launch point to any

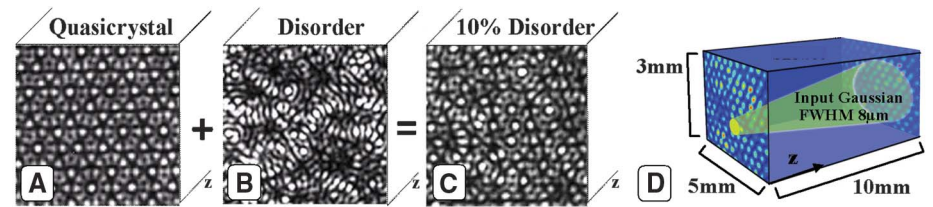


Fig. 1. Experimental scheme for transverse localization in photonic QCs containing disorder. A narrow optical beam is launched at the input face of a 2D quasicrystal lattice (A) containing disorder (B and C). The output intensity (D) pattern is monitored and ensemble-averaged over many realizations of disorder. FWHM, full width at half maximum.

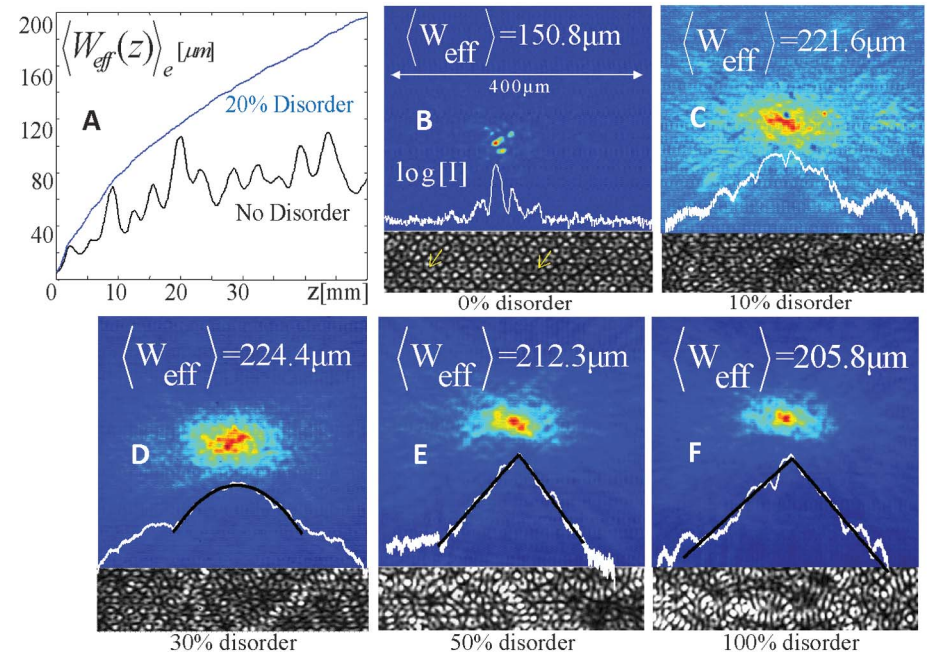


Fig. 2. Experimental and simulation results of transport through photonic quasicrystals, demonstrating disorder-enhanced transport and Anderson localization. (A) Simulation: beam width (ensemble-averaged) versus propagation distance for a pure QC (black) and a QC containing 20% disorder (blue). Transport is always higher in the disordered QC. (B to F) Experimental results: output intensity after $z = 10$ mm (ensemble-averaged), the log of its cross section (shown in white), and samples of the refractive-index profile (below each panel). Disorder-enhanced transport is apparent from the transition from 0% to 10% disorder [(B) and (C)]. For higher disorder levels, parabolic and linear fits [in (D) to (F)] indicate diffusive-like transport and the transition to Anderson localization, respectively. Yellow arrows in (B) indicate points of local 10-fold symmetry.

other center of local 10-fold symmetry yields virtually identical results. The experimental results are depicted in Fig. 2, B to F. Figure 2B shows the beam exiting the pure QC after 10 mm of propagation. The exiting beam, which has a mean width (W_{eff}) of ~ 150 μm , is always fractured and varies depending on the launch point, because the QC has no translational symmetry. Figure 2, C to F, depicts the ensemble-averaged output beam and its log-plot cross section, for increasing strength of disorder. The ensemble averaging is taken over 100 realizations of the disorder for Fig. 2, B to D, and over 50 realizations for Fig. 2, E and F, for each value of disorder strength. At 30% disorder (Fig. 2D), the wave packet is diffusive-like, as indicated by the parabolic cross section near the center of (the log of) the ensemble-averaged beam. A parabolic fit to these data in the central region, where the signal is strongest, gives a R^2 goodness-of-fit value of 96% (as shown in the figure). Notice that the width of the averaged beam in Fig. 2, C and D (221.6 and 224.4 μm , with standard deviations of 47 and 31 μm , respectively) is greater than the width of the output beam in the pure QC (150.8 μm in Fig. 2A), indicating enhanced transport. On the other hand, further increasing the disorder strength (50 and 100% disorder) makes the beam more localized (Fig. 2, E and F), while displaying the exponential tails characteristic of the transition to Anderson localization, with an average beam width of 212 and 206 μm (standard deviations of 28 and 35 μm), respectively. The linear fits to Fig. 2E give R^2 values of 96% (for both the left and right sides); the fits in Fig. 2F

gives R^2 values of 86 and 98% (left and right sides). The distribution of W_{eff} in all these experiments shows no significant outliers on the high end, meaning that the decay properties of the wave functions in Fig. 2, B to F, are not skewed by a small number of measurements.

Our results on disorder-enhanced transport call for a direct comparison between crystals and quasicrystals. We therefore simulate transport in triangular and QC lattices of the same mean lattice spacing. Figure 3A shows the simulated W_{eff} exiting the hexagonal lattice after propagating in it ($z = 30$ mm), as function of disorder strength. Clearly, for the hexagonal lattice, transport decreases monotonically with increasing disorder strength. In sharp contrast, for the QC lattice (Fig. 3B) increasing disorder first enhances transport, and only after reaching a pronounced peak transport begins to decline with increasing disorder. To examine the expansion rate of the beam, we follow W_{eff} while propagating through the QC (Fig. 3C) for different levels of disorder and calculate the derivative of $\log(W_{\text{eff}})$ with respect to $\log(z)$ to deduce its characteristic exponent. Figure 3D reveals that the exponent of the expanding beam, for a wide range of disorder levels, is close to 0.5, indicating a diffusive-like expansion. Increasing the disorder past the level causing maximal transport (between 5 and 10% in Fig. 3B) shows that the exponent converges toward 0.5. By fitting W_{eff} versus z , the diffusion constants for 10, 30, 50, and 100% disorder are found to be 0.038, 0.029, 0.029, and 0.027 μm , respectively, giving mean free paths of 8.1, 6.3, 6.3, and 5.7 μm [derived as in (3)]. As clearly

shown in Fig. 3C, the wave-packet widths greatly exceed these mean free paths, indicating that we are in the multiple-scattering regime. This fact, together with the characteristic exponent of 0.5, strongly suggests diffusive-like transport. We examine the log of the ensemble-averaged and azimuthally averaged beam intensities (over 100 realizations) in Fig. 3, E to H, as a function of the transverse radial coordinate, r . In these plots, for 0, 10, 30, and 50% disorder, respectively, we fit parabolas (Gaussian wave packets; i.e., diffusion) or lines (exponential wave packets; i.e., a signature of localization) only where the fit is highly appropriate ($R^2 > 97\%$). We find that for 10% disorder (Fig. 3F), some features of the original QC remain; thus, parabolic and linear fits are not appropriate. For 30% disorder (Fig. 3G), a Gaussian wave packet is observed at $z = 5$ mm, whereas the wave packet shows the exponential tails signifying the start of localization by $z = 20$ mm. At 50% disorder (Fig. 3H), the wave packet quickly reaches exponential decay. As explained below, we use a narrow beam selected specifically to excite pseudogap states to demonstrate disorder-enhanced transport [unlike in (3) where a broader beam was used]. Consequently, the beam is a superposition of many eigenmodes, some of which have high energy and extremely large localization lengths, larger than the simulation box. Therefore, we do not observe the wave function coming to an absolute halt. It is well known that in two dimensions, some localization lengths can be extremely large and out of reach of any simulation. That said, the beam in Fig. 3, G and H, exhibits exponential decay (for $z \geq 10$ mm),

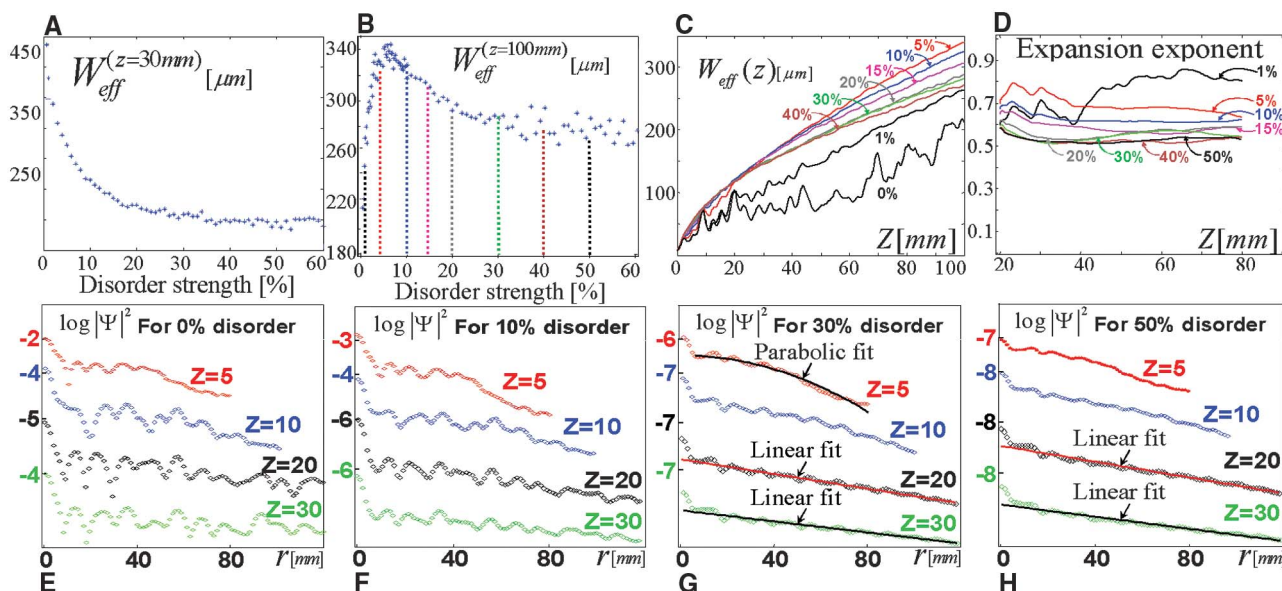


Fig. 3. Simulation results comparing transport through a hexagonal lattice and a QC for increasing disorder, showing disorder-enhanced and diffusive-like transport, as well as signatures of Anderson localization. (A and B) Ensemble-averaged beam width versus disorder strength for the hexagonal and QC lattices (same characteristic lattice spacing), showing disorder-enhanced transport for weak disorder (0 to 10%), then transport declines with further increase of disorder. (C) Beam width versus propagation distance in the

QC for disorder levels indicated in (B). (D) The derivative with respect to $\log(z)$ of the log-log plot of (C); i.e., the characteristic expansion exponent. Convergence to 0.5 with increasing disorder indicates diffusive-like transport. (E to H) Logarithm of the ensemble- and azimuthally averaged wave function at 0, 10, 30, and 50% disorder, for various propagation distances. Parabolic fits indicate diffusive-like transport, and linear fits indicate the transition to Anderson localization for sufficiently large distances and disorder levels.

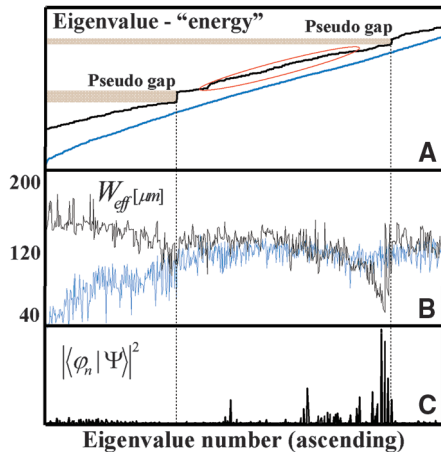


Fig. 4. Revealing the mechanism underlying disorder-enhanced transport in quasicrystals. **(A)** Band structure of a pure QC (black) and of a QC containing 20% disorder (blue). The fractured self-similar structure of the spectrum characteristic of a pure QC is smeared by the disorder, leading to a continuous density of states, which increases transport. The red oval denotes the fractal-like band. **(B)** W_{eff} of each eigenstate of a pure QC state (black) and in the QC containing 20% disorder (blue). Adding 20% disorder to a pure QC results in the broadening of all eigenfunctions associated with the vicinity of a large pseudogap (A). It is therefore expected that the expansion rate of a wave packet made up of such eigenfunctions will be higher in the disordered QC than in the pure QC. **(C)** Projection of a Gaussian wave packet (of Fig. 1A) on the eigenfunctions of the pure QC; ϕ_n represents the eigenfunctions. This wave packet, associated with the vicinity of a pseudogap, expands faster in a disordered QC, due to both the increase in the density of states (A) and to the broadening of the width, W_{eff} , of the eigenfunctions (B) in this region.

which is a clear signature that the wave packet is undergoing a transition to Anderson localization (1). The experimental and theoretical results displayed in Figs. 2 and 3 unequivocally show that disorder enhances transport in QCs; they demonstrate diffusive-like transport and show exponentially localized wave packets, a signature of the transition to Anderson localization.

These results raise the natural question: What is the underlying mechanism responsible for disorder-enhanced transport in QCs? Transport in atomic crystals is closely related to the density of states around the Fermi energy. In aperiodic systems, higher density of states is generally associated with broader eigenfunctions, which support higher transport. This is the case for potentials where the eigenmodes are localized (as in any potential containing disorder) or for critical states (as in QCs). To examine this point in our system, we solve Eq. 1 for a QC potential and find its eigenfunctions and eigenvalues β . Figure 4A shows a comparison between the band structure of a pure QC (black) and a QC containing 20% disorder (blue). The eigenvalues (energies) are presented in ascending order, because the notion of Brillouin

zone does not exist for a QC. Nonetheless, there are regions (pseudogaps) in the band structure (gray in Fig. 4A) where the density of states is considerably lower. The band structure of the quasiperiodic potential is fractal-like; hence, higher-order pseudogaps exist on any scale (12). It is this fractured structure that is responsible for the low density of states, especially around pseudogaps, which in turn leads to highly localized states and thus to low conductivity/transport in QCs.

When disorder is introduced in a QC, the highly localized states near the pseudogap couple to one another, together forming eigenstates that are broader and less localized (at other energies, disorder acts to “smooth out” the fractal band structure, but the effect on transport is less pronounced). In other words, disorder mediates “hopping” between localized quasicrystalline eigenstates near the pseudogap (30). In turn, this coupling between localized states results in smoothing of the density of states, reducing the pseudogap until it altogether disappears (blue curve in Fig. 4A).

We calculate and plot W_{eff} of each of the eigenfunctions (Fig. 4B) for pure QC (black) and for the QC containing 20% disorder (blue). Going back to the plot of the eigenvalues (Fig. 4A), we find that the eigenstates near the two large pseudogaps tend to be more localized (Fig. 4B). At the same time, simple initial wave functions (e.g., Gaussian) are found to easily excite the localized eigenstates near the higher pseudogap, an experimentally indispensable condition. Figure 4B shows that adding 20% disorder to a pure QC results in the broadening of all eigenfunctions with energies in the vicinity of a large pseudogap. It is therefore expected that the expansion rate of a wave packet made up of such eigenfunctions will be higher in the disordered QC than in the pure QC. That is, the phenomenon of disorder-enhanced transport is expected to be most pronounced for wave packets associated with the pseudogaps in QCs. With this in mind, we analyze the wave packets launched in our simulations and experiments and examine their transport. Consider the Gaussian wave packet of Fig. 2A whose propagation displays enhanced transport in the disordered QC. Figure 4C displays the projection of this wave packet on the eigenfunctions of the pure QC. The underlying mechanism for disorder-enhanced transport in QCs is therefore due to the increase in the density of states near its pseudogaps. This explanation holds well for any launch point of a high 10-fold symmetry, which always excites mostly localized states from the vicinity of the pseudogap. Finally, we emphasize that the Fermi energy in atomic QCs resides in a pseudogap (similar to crystals where it resides in the gap), hence our initial wave packet represents conduction electrons residing in a $k_B T$ -sized stripe (where k_B is the Boltzmann constant and T is temperature) around the Fermi energy.

This article was devoted to providing a direct experimental demonstration that transport in quasicrystals is enhanced by virtue of disorder, while displaying features associated with diffusion

and localization. We studied this fundamental phenomenon and elucidated its origins, relating it to the basic properties of quasicrystalline media in the presence of disorder.

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29. For each realization of disorder, the confinement of the beam at the output plane is quantified by the inverse participation ratio $P = \frac{[\int I(x,y,L)^2 dx dy]}{[\int I(x,y,L) dx dy]^2}$ (where I is the intensity and L is the propagation distance), having units of inverse-area and an average effective width $W_{\text{eff}} = \langle P \rangle^{-1/2}$; the averaging is over multiple realizations of the disorder (same statistics).
30. We emphasize that the disorder-enhanced transport in QCs observed here is fundamentally different from transport effects associated with the Urbach tail. See detailed discussion in (27).

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Fig. S1

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Net Oxidative Addition of C(sp³)-F Bonds to Iridium via Initial C-H Bond Activation

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Carbon-fluorine bonds are the strongest known single bonds to carbon and as a consequence can prove very hard to cleave. Although vinyl and aryl C-F bonds can undergo oxidative addition to transition metal complexes, this reaction has appeared inoperable with aliphatic substrates. We report the addition of C(sp³)-F bonds (including alkyl-F) to an iridium center via the initial, reversible cleavage of a C-H bond. These results suggest a distinct strategy for the development of catalysts and promoters to make and break C-F bonds, which are of strong interest in the context of both pharmaceutical and environmental chemistry.

The oxidative addition of single-bonded substrates to transition metal centers and the microscopic reverse, reductive elimination, compose an especially useful reaction class (Scheme 1).

The addition and elimination of bonds to carbon are key steps in the majority of organic reactions catalyzed or promoted by metal complexes. In this context, the addition of carbon-halogen bonds, specifically C-Cl, C-Br, and C-I, has been studied and exploited extensively over the past five decades (1–6). The formation and cleavage of carbon-fluorine bonds is of great interest in medicinal and environmental chemistry (7–14); for example, about 20% of pharmaceuticals and 40% of agrochemicals have C-F bonds (8). Examples of oxidative addition and reductive elimination of C-F bonds, however, are essentially limited to those with aryl and vinyl carbon atoms (7, 14–17). We report the oxidative addition of C(sp³)-F bonds to a transition metal center. These reactions do not proceed via direct (3-centered) C-F addition, nor by any of the several pathways that have previously been proposed for carbon-halogen oxidative addition (1–6); instead, the C(sp³)-F additions take place via initial oxidative addition of a C(sp³)-H bond.

We recently reported that the pincer-ligated iridium complex (PCP)Ir {where PCP is κ^3 -C₆H₃-2,6-[CH₂P(*t*-Bu)₂]₂; *t*-Bu indicates a tert-butyl group} undergoes oxidative addition of C(sp³)-O bonds (18). Our study was limited to aryloxy-carbon bonds (ArO-C), which have homolytic bond dissociation energies less than 65 kcal/mol [e.g., 64 kcal/mol for CH₃-OPh (19)]. In contrast, fluorine forms the strongest known bonds to sp³-hybridized carbon; for example, the H₃C-F bond dissociation energy is 110 kcal/mol (19). Nonetheless, because we found that more electronegative OR groups [e.g., R is 3,5-(CF₃)₂C₆H₃]

avored C-OR addition to (PCP)Ir (18), we considered that C-F addition to the same fragment might be possible as well.

(PCP)Ir(NBE) (1; NBE is norbornene) acts as a precursor to the active fragment (PCP)Ir (20). When fluoromethane (1 atm) was added to a *p*-xylene-*d*₁₀ solution of 1, ³¹P nuclear magnetic resonance (NMR) spectroscopy revealed complete conversion to a single major product [2; chemical shift (δ) 50.4 parts per million (ppm), doublet, coupling constant (²J_{FP}) = 10.5 Hz; yield by NMR spectroscopy >95%] within 20 min at 50°C (Fig. 1) (21). In benzene-*d*₆ solvent, product 2 is characterized by a triplet at δ -28.20 ppm (²J_{PC} = 4.3 Hz) in the ¹³C NMR spectrum and a signal at δ 1.85 ppm (Ir-CH₃) in the ¹H NMR spectrum, which appears as a broad singlet at room temperature and as a doublet of triplets at 80°C (³J_{PH} = 4.7 Hz, ³J_{FH} = 3.5 Hz). When ³¹P-decoupling is applied, this ¹H NMR signal appears as a doublet (³J_{FH} = 3.5 Hz) (fig. S2). These ¹H and ¹³C NMR signals are characteristic of a methyl group coordinated to iridium at the apical site of a square pyramidal structure (18, 22). Accordingly, the ¹H NMR spectrum indicates a PCP ligand with only C_s symmetry, while two-dimensional (2D) nuclear Overhauser effect spectroscopy (NOESY) spectra show that the putative methyl ligand interacts with only one set of phosphino-*t*-butyl groups.

The ¹⁹F NMR spectrum shows a broad signal at δ -252.6 ppm (peak width at half-height, *W*_{1/2} = 28.6 Hz) at 25°C; both the broadness and chemical shift are consistent with that of other iridium fluoride complexes, in particular coordinatively unsaturated complexes (23, 24). Although the width of the ¹⁹F NMR signal precluded direct observation of the ³¹P-¹⁹F coupling that was observed in the ³¹P NMR spectrum (²J_{FP} = 10.5 Hz), application of ³¹P-decoupling (¹⁹F{³¹P} NMR) resulted in a decrease in *W*_{1/2} from 28.6 Hz to 13.9 Hz (fig. S2), confirming the existence of ³¹P-¹⁹F coupling. Simulation of the ¹⁹F NMR signal using Spinworks software (21) successfully reproduced the signal (with and without ³¹P decoupling) on the basis of coupling to two ³¹P

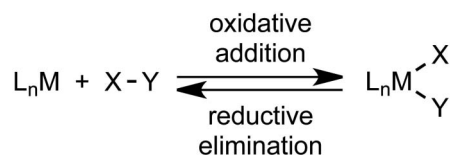
nuclei with ²J_{PF} = 10.5 Hz, coupling to three ¹H nuclei with ³J_{HF} = 3.5 Hz, and a linewidth of 10.0 Hz (figs. S4 and S5).

When the reaction of Fig. 1 was conducted with ¹³C-labeled methyl fluoride, the major signal in the ¹H-decoupled ¹³C NMR spectrum [¹³C(¹H)] was a triplet at δ -28.20 ppm (²J_{PC} = 4.4 Hz); without ¹H decoupling, this signal appeared as a quartet of triplets (¹J_{HC} = 136 Hz, ²J_{PC} = 4.4 Hz) because of coupling with three protons. The ¹H-¹³C coupling of 136 Hz was also manifest in the ¹H NMR signal at δ 1.85 ppm. The assignment of these signals to an iridium-bound methyl group derived from methyl fluoride is thus confirmed. The ²J_{PC} coupling was also manifest in the ³¹P NMR signal, which appeared as a partially resolved doublet of doublets at δ 50.4 ppm that could be simulated (Spinworks) with coupling constants ²J_{FP} = 10.5 Hz and ²J_{PC} = 4.4 Hz and a linewidth of 4.5 Hz (fig. S6) (21).

The results of elemental analysis and LIFDI (liquid introduction field desorption ionization) mass spectrometry (25) of 2 (fig. S1) were fully consistent with the characterization of 2 as (PCP)Ir(CH₃)F (21).

Evaporation of a pentane solution of 10 mg of 2 gave only a few small crystals (<10 μg each). The x-ray structure of a crystal in which complex 2 is cocrystallized with another iridium complex, apparently a hydrolysis product presumably resulting from the presence of adventitious water (26–28), was determined to the extent that 2 was clearly identified. However, the molecular structure details of that cocrystal are quite imprecise because of substantial disorder for all molecules of that structure and are therefore not included here. Under more rigorously dry conditions, including the use of silylated glassware, the spectroscopic purity of the product solution was greater, but the purer solutions did not yield x-ray-quality crystals.

Derivatives of benzyl fluoride also reacted with (PCP)Ir. The reaction of 1 with 1.0 equiv of 3a or 3b was complete within 1 hour at 60°C as determined by ³¹P NMR spectroscopy (Fig. 2; steric crowding by the methyl or trifluoromethyl groups at the meta positions of the aryl ring prevents C-H addition at the ortho and para aryl positions, which would otherwise occur in preference to addition of the C-F bonds). Benzyl fluoride derivative 3b cleanly gives 4b in high yield (95% by NMR), whereas the reaction with 3a affords lower yields (~70%) along with unidentified by-products. 4a and 4b were characterized as the C-F addition products by NMR methods (21). Crystals of 4b suitable for x-ray structure determination were obtained from a pentane solution



Scheme 1.

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at -40°C (Fig. 3). The coordination geometry of **4b** was determined to be a slightly distorted square pyramid, distinguished by an acute C–Ir–C angle $[78.93(19)^{\circ}]$ (21), which is characteristic of L_2IrXY_2 species (L is any charge-neutral ligand) where X^- is a π donor and weak σ donor and Y^- is a strong σ donor and weak π donor (29). Bond distances and angles are otherwise unexceptional. Terminal Ir–F bond lengths have been reported to range from 2.00 Å to 2.20 Å for Ir(III) complexes, with one example of a structurally characterized five-coordinate Ir(III) fluoride complex, $(\text{Ph}^t\text{Bu}_2\text{P})_2\text{IrH}_2\text{F}$ (where Ph is a phenyl group) (23), in which the Ir–F bond length is 2.045 Å. In good agreement with these values, the Ir–F bond length in **4b** was determined to be 2.074(3) Å.

We have previously shown that oxidative addition of the methyl-oxygen bond of anisole derivatives ($\text{H}_3\text{C}-\text{OAr}$) occurs via initial addition of a methyl C–H bond to (PCP)Ir as evidenced by a substantial normal CH_3/CD_3 kinetic isotope effect (KIE) (18). When **1** was treated with a large excess of 3,5-bis(trifluoromethyl)benzylfluoride and its deuterated (CD_3F) analog (at least fivefold excess of each), a $\text{CH}_2\text{F}/\text{CD}_2\text{F}$ kinetic isotope effect of 2.7(3) at 60°C was determined (21), implying that C–H bond activation is involved in or occurs before the product-determining step in the oxidative addition of the $\text{C}(\text{sp}^3)\text{--F}$ bond. In analogy with the mechanism of C–O bond activation in anisole derivatives, we propose that initial C–H bond activation is followed by α -fluorine migration (30) to generate an intermediate methylidene complex (**5**); iridium-to-methylidene hydride migration then completes the net C–F oxidative addition (Fig. 4A) (31, 32).

The proposed intermediates shown in Fig. 4A, the $\text{sp}^3\text{--C--H}$ bond addition product and the methylidene complex **5**, are apparently too unstable to be observed, as might be expected, but we hypothesized that the stability of both would be increased by the presence of additional fluorine substituents on the alkyl (33) and carbene (34, 35) groups, respectively. Accordingly, we investigated the reaction of CHF_3 with **1**. Trifluoro-

methane (1 atm) was added to a frozen methyl *t*-butyl ether solution of **1** at -196°C . Monitoring the reaction at -10°C revealed slow conversion to an intermediate, **6**, with a triplet at δ 7.7 ppm (t , $J_{\text{PF}} = 8.0$ Hz) in the ^{19}F NMR spectrum and a broad singlet at 67.7 ppm in the ^{31}P NMR spectrum. The ^1H NMR spectrum of **6** showed a broad triplet indicative of a hydride at -45.5 ppm, implying that the complex is square pyramidal with the hydride occupying the apical position. The ^{19}F chemical shift at 7.7 ppm is in the range of values reported for CF_3 groups bound to iridium (28, 35) and other platinum group metals (12, 30). All data are thus consistent with the assignment of **6** as the product of trifluoromethane C–H bond oxidative addition (Fig. 4B). When the temperature was increased to 20°C , formation of the four-coordinate difluorocarbene complex $(\text{PCP})\text{Ir}=\text{CF}_2$ (**8**, 60% yield) was observed in place of the six-coordinate carbene complex **7** that would be the expected analog of **5**. Although **8** was not isolated, NMR spectroscopy unambiguously supports its structural assignment. A triplet appears in the ^{19}F NMR spectrum at δ 95.1 ppm ($^3J_{\text{PF}} = 14.6$ Hz), and a triplet of triplets appears in the ^{13}C NMR spectrum at 205.3 ppm with a large $^1J_{\text{FC}}$ value ($^1J_{\text{FC}} = 437$ Hz, $^2J_{\text{PC}} = 8.8$ Hz); these spectroscopic features are highly characteristic of metal difluorocarbene complexes (30, 35). The ^1H NMR spectrum is indicative of a PCP ligand with effective C_{2v} symmetry. Along with complex **8**, $(\text{PCP})\text{Ir}(\text{CO})$ (10%) and an unidentified PCP-containing product (30%) are observed from the reaction with CHF_3 (21). The reaction of adventitious water with difluorocarbene complexes to give the corresponding carbonyl complexes is well known and is presumably responsible for the formation of $(\text{PCP})\text{Ir}(\text{CO})$ (10, 34, 35). Presumably, complex **8** results from loss of H–F from **7**. Accordingly, a broad singlet was found in the ^1H NMR spectrum at δ 12.1 ppm; this chemical shift value is strongly diagnostic of transition metal complexes of bifluoride (36), thus shedding some light on the nature of the minor product, although it remains to be characterized.

The results of density functional theory (DFT) calculations (Fig. 5 and table S1) (21) offer further support for the proposed mechanism shown in Fig. 4A. Direct addition of the C–F bond of CH_3F (i.e., addition via a three-centered transition state) is calculated to have a prohibitively high barrier [standard Gibbs energy of activation ($\Delta G^\ddagger$) of 31.1 kcal/mol relative to the free (PCP)Ir fragment and CH_3F or 37.5 kcal/mol relative to the (PCP)Ir(NBE) starting complex. On the basis of the approximate reaction rates, the experimental free energy barrier is about 23 kcal/mol. In contrast, the pathway of Fig. 4A is calculated to have a barrier $\Delta G^\ddagger = 16.5$ kcal/mol relative to free (PCP)Ir or 22.9 kcal/mol relative to (PCP)Ir(NBE) (fully consistent with the observed rate), attributable to the transition state of an α -fluorine-migration rate-determining step (Fig. 5). Thus, if secondary isotope effects are neglected, the observed KIE is expected to be equal to the equilibrium isotope effect (EIE) for C–H/D addition. The competition KIE of 2.7(3) observed with the substrate bis(trifluoromethyl)benzylfluoride (Fig. 2) is indeed well within the range expected of such EIEs (37, 38).

Alkyl fluorides in which the alkyl group has a β -hydrogen atom also react with (PCP)Ir to cleave the C–F bond but in a completely different manner. For example, addition of fluoroethane to a *p*-xylene- d_{10} solution of **1** results in an immediate color change from dark red to brown, and ^{31}P NMR spectroscopy reveals the formation of an equimolar mixture of $(\text{PCP})\text{Ir}(\text{H})(\text{F})$, **9**, and the known complex $(\text{PCP})\text{Ir}(\text{ethylene})$, **10** (Fig. 6) (39). The hydride ligand of complex **9** resonates at δ -35.4 ppm in the ^1H NMR spectrum as a doublet of triplets ($^2J_{\text{FH}} = 30.5$ Hz, $^2J_{\text{PH}} = 12.1$ Hz), implying an approximately square pyramidal structure with hydride at the apical position. In the ^{19}F NMR spectrum, the Ir–F resonance appears as a broad doublet at δ -228 ppm ($J_{\text{HF}} = 30.1$ Hz). In contrast to the reaction with fluoroethane, addi-

Fig. 1. Conversion of (PCP)Ir(NBE) to **2** by addition of fluoromethane.

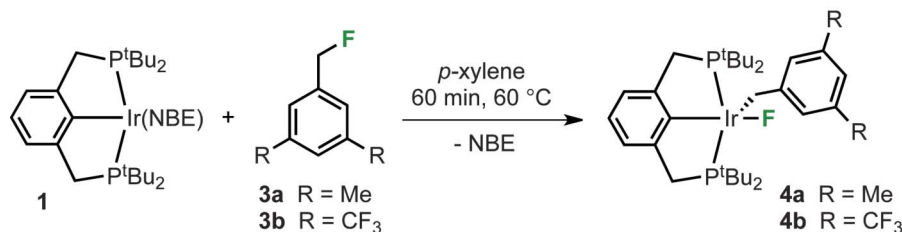
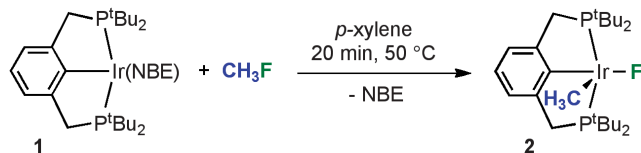


Fig. 2. The reaction of **1** with 1.0 equiv of **3a** or **3b**.

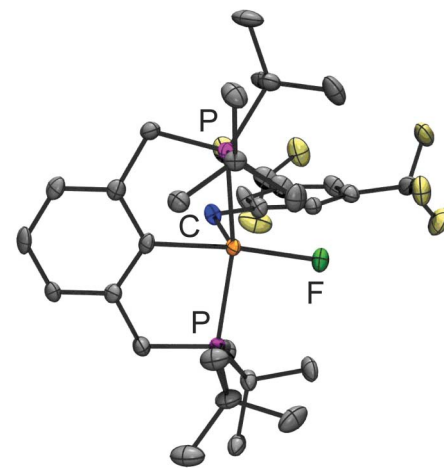


Fig. 3. X-ray crystal structure of complex **4b**. Oak Ridge thermal ellipsoid plot (ORTEP) drawing with atoms at 50% probability; hydrogen atoms omitted for clarity.

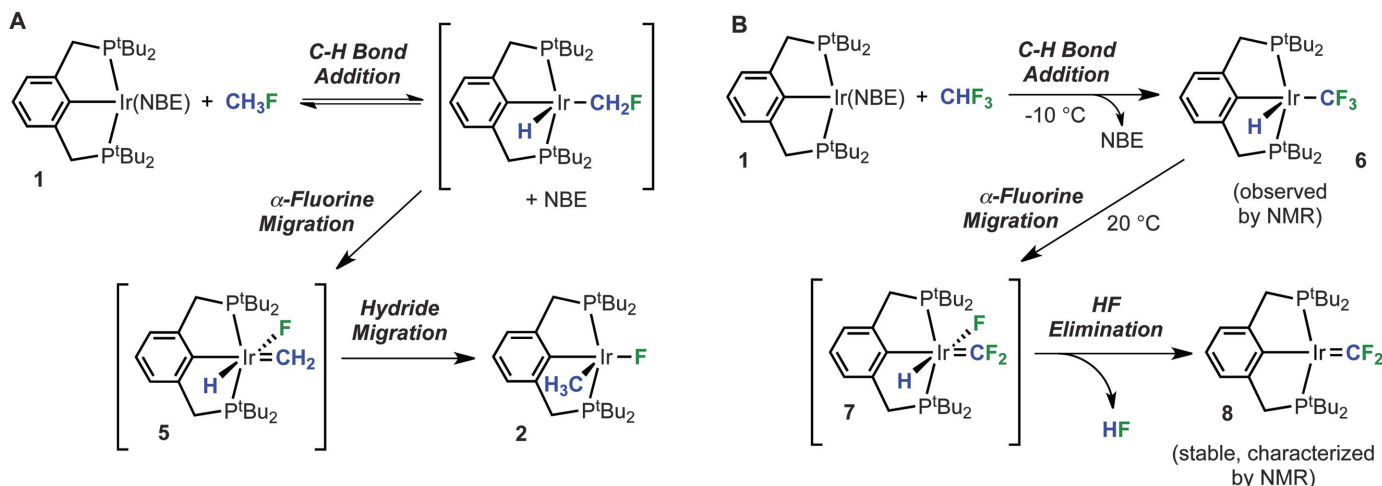


Fig. 4. (A) Proposed mechanism for oxidative addition of CH_3F to $(\text{PCP})\text{Ir}$. (B) Reaction of **1** with trifluoromethane.

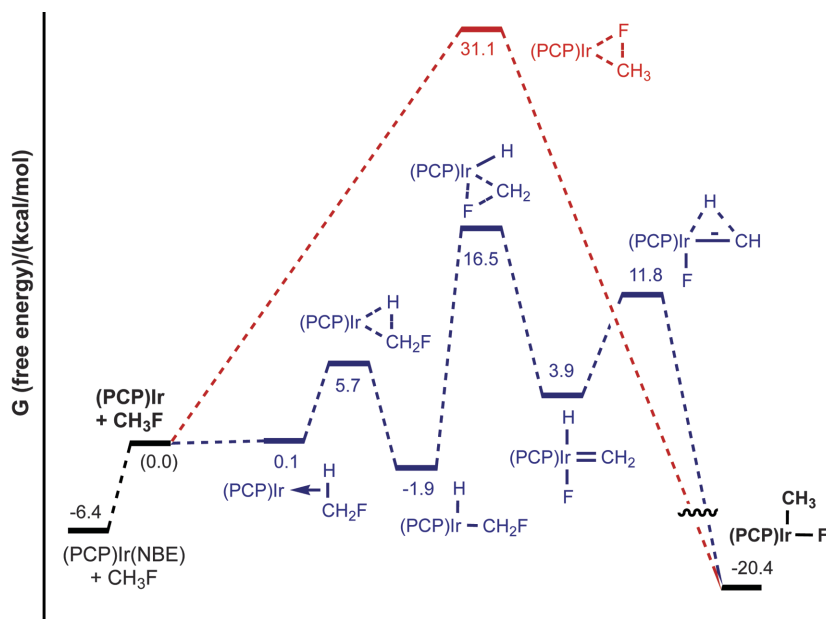


Fig. 5. Free energy diagrams pertaining to the proposed pathway for the reaction of $(\text{PCP})\text{Ir}$ with CH_3F (blue) and to direct C-F addition (red).

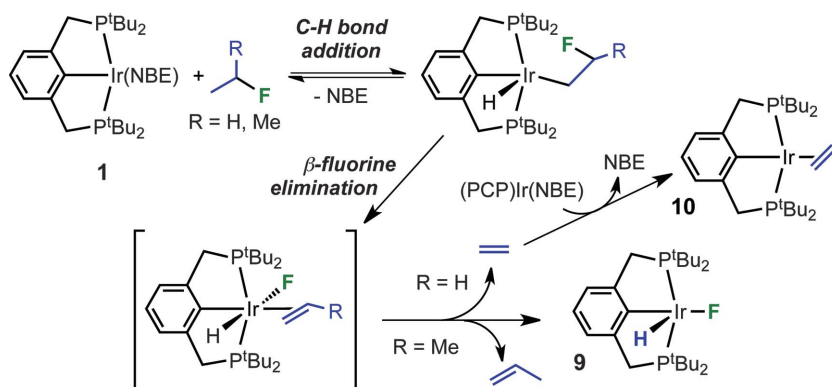


Fig. 6. Proposed mechanism for dehydrofluorination of ethyl fluoride and isopropyl fluoride by $(\text{PCP})\text{Ir}$.

tion of 2-fluoropropane to a *p*-xylene solution of **1**, followed by heating at 80°C for 15 min, affords a quantitative yield of complex **9**. The difference between the reactions of fluoroethane and 2-fluoropropane is presumably attributable to the stronger binding ability of ethylene relative to propene. We propose (Fig. 6) that these reactions proceed via addition of the β -C-H bond (in contrast to the reaction with CH_3F) followed by β -F migration (40); an analogous pathway has been proposed for the reaction of **1** with ethoxybenzene (18).

Considering the broader implications of these results, microscopic reversibility dictates that the lowest energy pathway for C-F elimination from **2** must proceed via initial α -H migration from carbon to the metal center. This unusual mechanism may offer insight applicable toward the development of methods for the formation of sp^3 -C-F bonds via C-F elimination from a metal center; such reactions are of substantial current interest in the context of the synthesis of pharmaceutically important compounds. The reactions of ethyl and isopropyl fluoride, substrates with C-H bonds that are β to C-F bonds, likewise have implications for both catalytic cleavage of hydrofluorocarbon C-F bonds and their formation via the hydrofluorination of olefins.

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Supporting Online Material

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References (41–67)

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Abiotic Pyrite Formation Produces a Large Fe Isotope Fractionation

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The iron isotope composition of sedimentary pyrite has been proposed as a potential proxy to trace microbial metabolism and the redox evolution of the oceans. We demonstrate that Fe isotope fractionation accompanies abiotic pyrite formation in the absence of Fe(II) redox change. Combined fractionation factors between Fe(II)_{aq}, mackinawite, and pyrite permit the generation of pyrite with Fe isotope signatures that nearly encapsulate the full range of sedimentary $\delta^{56}\text{Fe}_{\text{pyrite}}$ recorded in Archean to modern sediments. We propose that Archean negative Fe isotope excursions reflect partial Fe(II)_{aq} utilization during abiotic pyrite formation rather than microbial dissimilatory Fe(III) reduction. Late Proterozoic to modern sediments may reflect greater Fe(II)_{aq} utilization and variations in source composition.

The analysis and interpretation of textural, compositional, and isotopic signatures contained within sedimentary pyrite (FeS_2) inform debate concerning models for the evolution of Earth's ocean and atmosphere system. Over the past decade, growing interest in Fe isotopes as biogeochemical tracers has led to the collection of a spectrum of data that illustrates the variation of $\delta^{56}\text{Fe}$ for sedimentary pyrite throughout Earth's history, ranging between $\delta^{56}\text{Fe} \sim +0.2$ to -1 per mil (‰) in the Phanerozoic and $+1.2$ to -3.5 ‰ in the Paleoproterozoic and Archean (1, 2). In modern anoxic basins, diagenetic pyrite displays isotopic compositions of between -0.4 and -1.2 ‰ (3).

Detailed interpretations of the fluctuating secular $\delta^{56}\text{Fe}_{\text{pyrite}}$ trends are still debated because natural data are far from theoretical computations that predict an equilibrium fractionation $\Delta^{56}\text{Fe}_{\text{Fe(II)-pyrite}}$ varying from ~ -2.5 to -4.5 ‰, with pyrite being ^{56}Fe enriched (4). On mixing aqueous Fe(II) [$\text{Fe(II)}_{\text{aq}}$] and S(-II)_{aq}, the first phase to precipitate, mackinawite (FeS_m), incorporates lighter isotopes, recording a kinetic fractionation $\Delta^{56}\text{Fe}_{\text{Fe(II)-FeS}}$ varying from $+0.9$ to $+0.3$ ‰ (5), and it has been proposed that sedimentary pyrite formation should record a fractionation similar to $\Delta^{56}\text{Fe}_{\text{Fe(II)-FeS}}$ (1, 4, 6). This implies that a mechanism is required to produce ^{56}Fe isotope excursions more negative than -1 ‰ as recorded in Archean sedimentary rocks. Dissimilatory iron(III) reduction (DIR), an important anoxic metabolic pathway, can produce large amounts of ^{56}Fe -depleted Fe(II)_{aq}, and it has been proposed that the large Fe isotope variations observed in Precambrian sedimentary pyrite and banded iron formations (BIFs) are evidence for widespread DIR (7). This potential microbial origin for the ^{56}Fe depletion of Archean pyrite

has been supported by covariations in Fe and S isotopes (8). However, such large variations in $\delta^{56}\text{Fe}$ have not been identified in modern anoxic sediments, where these microbial processes are substantial (3, 9). Because abiotic Fe(II)_{aq} oxidation also produces large fractionations where residual Fe(II)_{aq} is ^{56}Fe -depleted (10), redox effects have been proposed as an alternative explanation for Paleoproterozoic and Archean $\delta^{56}\text{Fe}_{\text{pyrite}}$ (2). Fe removal as Fe (oxy)hydroxides and BIFs precursor minerals would preferentially incorporate ^{56}Fe (2, 11), and subsequent sulfide precipitation would reflect the ^{56}Fe -depleted Fe(II)_{aq}. Interestingly, both of these models are predicated on the hypothesis that pyrite is a passive recorder of the Fe(II)_{aq} pool. The assumption is that an isotopically light Fe reservoir is the essential ingredient to produce isotopically light pyrite.

We report experimentally derived Fe isotope fractionation factors for abiotic pyrite formation at 40°C and 100°C at $\text{pH} = 6$ (12). Pyrite was synthesized under anoxic conditions via the H_2S pathway (13) where FeS_m (initial $\delta^{56}\text{Fe}_{\text{FeS}}$ was $+0.3$ ‰) dissolves to form aqueous FeS clusters (FeS^0_{aq}), which react with H_2S to form pyrite. Pyrite was separated from its Fe(II) reservoir [$\text{Fe(II)}_{\text{RES}} = \text{FeS}_m + \text{FeS}^0_{\text{aq}}$], and the isotopic compositions of pyrite and Fe(II)_{RES} were measured. At 40°C and 100°C , the abiotic fractionation, $\Delta^{56}\text{Fe}_{\text{Fe(II)RES-pyrite}}$, varies from $+1.7$ to $+3.0$ ‰ $\pm$ 0.1 ‰ (2 SD) (-2.2 ‰ on average) (Fig. 1A). Isotopic mass balances for each experiment (table S1) and replicate analysis indicate that the experimental error is small and that our results are reproducible. The kinetic fractionation factors [$\alpha'_{\text{Fe(II)RES-pyrite}}$] are 1.0025 ± 0.0007 and 1.0021 ± 0.0004 at 40°C and 100°C , respectively. Within errors, these fractionation factors are indistinguishable, and on average $\alpha'_{\text{Fe(II)RES-pyrite}}$ is 1.0022 ± 0.0007 . This is large compared with the fractionation during FeS_m formation. We measured maximum rates of pyrite formation ($\sim 2.8 \times 10^{-6}$ mol of

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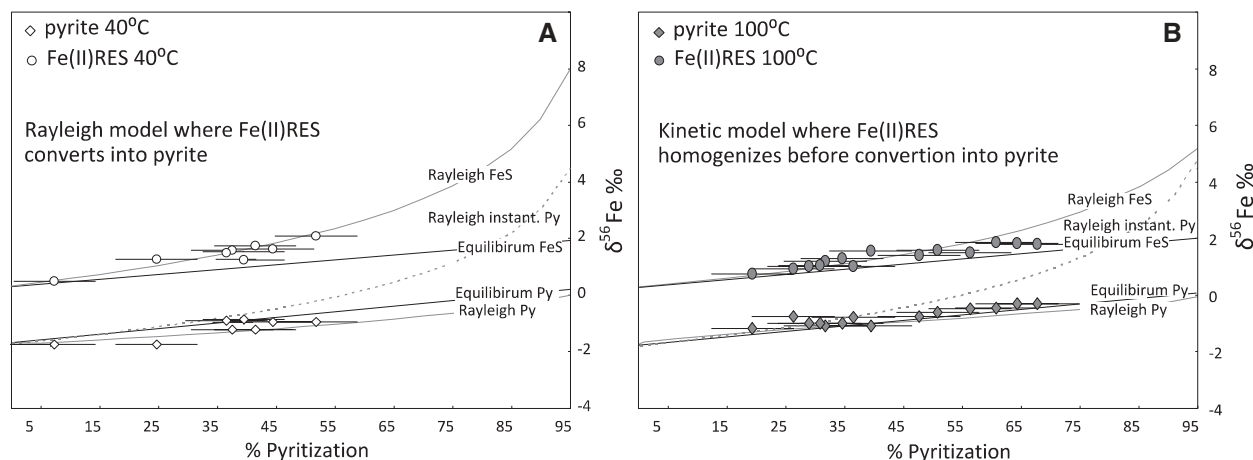


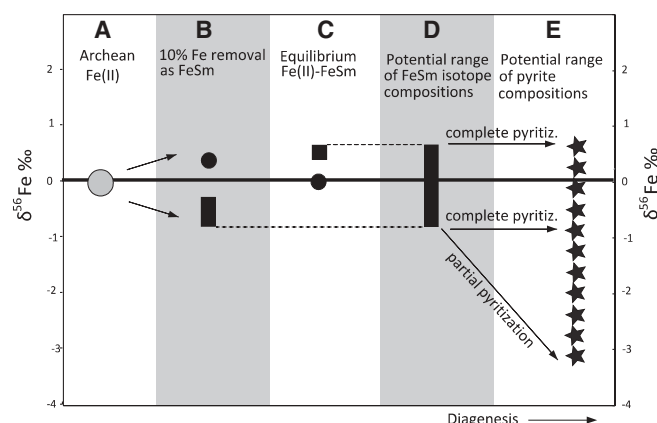
Fig. 1. Results for 40°C [(A) white symbols] and 100°C [(B) gray symbols] experiments. Circles and diamonds represent Fe(II)_{RES} and pyrite, respectively. Bold lines represent a putative isotope equilibrium evolution. Dashed lines ("Rayleigh instant. Py") represent the composition of neoformed pyrite at every time point, and the bulk compositions of pyrite and Fe(II)_{RES} evolve along the light curves. Both data sets fit a kinetic model (Rayleigh model,

light curves). At 40°C, Fe(II)_{RES} evolves more quickly toward ^{56}Fe -enriched values because FeS^0_{aq} , from which pyrite precipitates, gets more and more ^{56}Fe -enriched without equilibration with FeS_m . At 100°C, FeS_m and FeS^0_{aq} are allowed to equilibrate before transferring into pyrite; therefore, Fe(II)_{RES} evolves more slowly toward ^{56}Fe -enriched values. Error bars indicate the 2 SD precision based on x-ray diffraction replicate analysis.

pyrite $\text{l}^{-1} \text{s}^{-1}$) close to published data [$\sim 3 \times 10^{-6}$ mol of pyrite $\text{l}^{-1} \text{s}^{-1}$ (13)]. The pyrite-forming process is mechanistically uniform over the 25° to 125°C temperature range (13), and the observed temperature-independent effect indicates that our results may be extrapolated with reasonable confidence to ambient temperatures. The potential Fe isotope effect associated with FeS_m dissolution into FeS^0_{aq} is unknown but should be small because FeS_m and FeS^0_{aq} are structurally congruent (14). This means that measured $\Delta^{56}\text{Fe}_{\text{Fe(II)RES-pyrite}}$ approximates $\Delta^{56}\text{Fe}_{\text{FeS}_m\text{-pyrite}}$ and $\Delta^{56}\text{Fe}_{\text{FeS(0)aq-pyrite}}$.

The $\delta^{56}\text{Fe}$ values for Fe(II)_{RES} and pyrite fit with a simple Rayleigh model for the experiments carried out at 40°C (Fig. 1A). In this model, Fe(II)_{RES} is progressively converted into pyrite in a closed system. At 100°C (Fig. 1B), the system apparently evolves along an isotopically equilibrated pathway. Pyrite is very sparingly soluble (15), and pyrite formation is effectively a unidirectional reaction; thus, the associated fractionation is very unlikely to reflect isotopic exchange equilibrium. Figure 1B shows a Rayleigh model that allows continuous FeS_m - FeS^0_{aq} equilibration within the reservoir during pyrite precipitation. The model suggests that our data can be reproduced by using a unidirectional, multistep pathway and that isotopic equilibrium is only apparent. Consequently our experimentally determined results differ considerably from theoretical computations that predict ^{56}Fe enrichment in pyrite at equilibrium (4). The fractionation recorded here is large compared with other reactions that do not involve any redox change to Fe(II) atoms (5). Although there is no redox change for Fe(II) during pyrite formation, there is a shift from Fe(II) in a tetrahedrally coordinated high-spin d^6 configuration in FeS^0_{aq} to Fe(II) in an octahedrally coordinated low-spin d^6 configuration in pyrite (15). Preferen-

Fig. 2. Schematic diagram representing the abiogenic formation of ^{56}Fe -depleted pyrite. The light gray circle (column A) represents the isotopic composition of Archean Fe(II)_{aq} seawater (20). Rectangles and black circles represent the isotopic compositions of FeS_m and the residual liquid, respectively. Gray stars represent the composition of sedimentary pyrite. The isotopic evolution of iron sulfides during diagenesis is shown during FeS_m precipitation [column B (5)], equilibration with Fe(II)_{aq} [column C (21)], and transfer into pyrite (column E, this study).



tial incorporation of ^{56}Fe -depleted Fe(II) into pyrite may be related to selection during this change of coordination and spin state (16) during pyrite formation. Abiotic Fe isotope fractionations induced by changes in geometry have been previously proposed (17), but it is not clear whether this is the case for iron sulfides.

In most sedimentary environments, the H_2S pathway is the dominant pyrite-forming mechanism because polysulfide is a minor $\text{S(-II)}_{\text{aq}}$ species relative to $\text{H}_2\text{S}_{\text{aq}}$ under the entire oceanic pH range (15). Polysulfides may be more important at the oxic/anoxic interface [e.g., at the surface of Fe(III) oxyhydroxides] under alkaline conditions, and the polysulfide pathway may nucleate pyrite. Further pyrite formation, however, would involve H_2S , and bulk pyrite signatures reflect the H_2S pathway (18). The role of FeS_m as a reactant for pyrite formation may have been particularly important in Proterozoic and Archean oceans, which

were characterized by high Fe(II)_{aq} concentrations (19). In anoxic Archean oceans, the precipitation of iron sulfides was restricted to regions where bacterial sulfate was actively producing S(-II) . Assuming continuous hydrothermal Fe(II)_{aq} inputs with an isotopic composition $\sim 0\text{‰}$ (7, 20), Fe(II) would be partly removed from solution as FeS_m isotopic compositions varying from -0.9 to -0.3‰ (5). FeS_m dissolution to FeS^0_{aq} and isotope exchange between FeS^0_{aq} and Fe(II)_{aq} would produce a range of isotopic compositions varying from -0.9 to $+0.5\text{‰}$ for FeS^0_{aq} (5, 21). Subject to the rate and the extent of Fe utilization, subsequent pyrite would record isotopic compositions from ~ -3.1 to $+0.5\text{‰}$ (Fig. 2). This means that, by combining our result for $\Delta^{56}\text{Fe}_{\text{FeS(0)aq-pyrite}}$ with kinetic and equilibrium Fe isotope fractionation between Fe(II)_{aq} and FeS_m , almost the entire range of natural pyrite compositions falls within the range of possible compositions

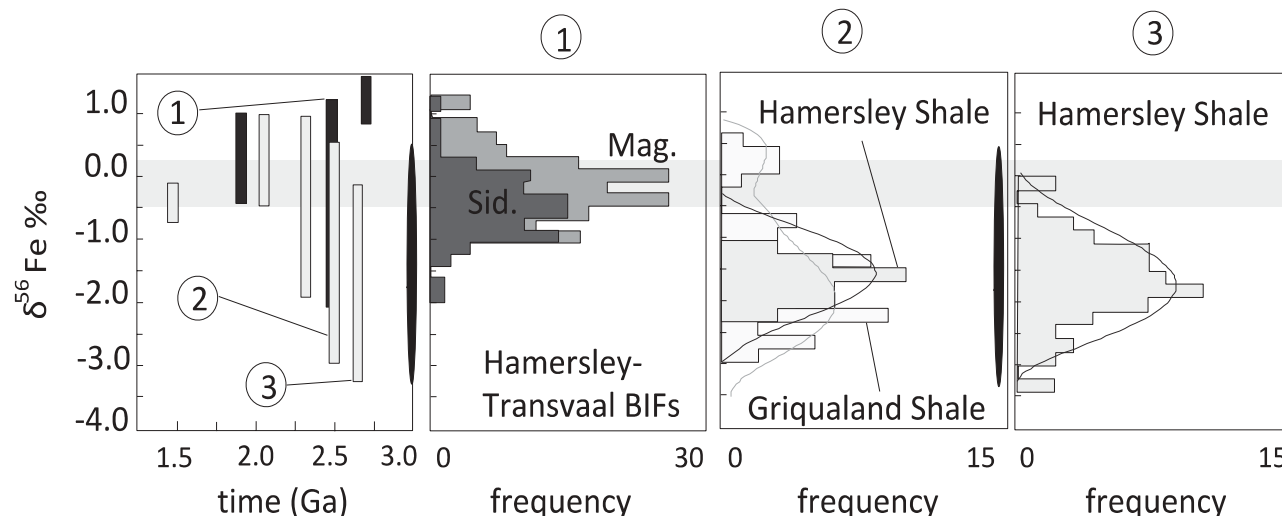


Fig. 3. The Fe isotopic distribution of some Archean BIFs minerals (magnetite and siderite) in the Hamersley and Transvaal basins (1) (left) and of some Archean sulfidic shales (pyrite and pyrite nodules) in the Hamersley (2 and 3) and Griqualand basins (2) (right). BIFs data are from (7). Pyrite data are from (2). The mean weighed value is ~ 0 to -0.5‰ for BIFs and $\sim -1.5\text{‰}$ for

Archean sedimentary pyrite. The black areas represent the possible spectrum of pyrite isotope signatures (this study) when pyrite forms from precipitated FeS_m that represents 10% of the $\text{Fe(II)}_{\text{aq}}$ reservoir. The gray area represents the composition range between igneous rocks and hydrothermal fluids. Modified after (7).

generated by the abiological pyrite formation process itself. Thus, Archean and Proterozoic Fe isotope excursions need not reflect particular redox or DIR contributions.

The idea that a large ^{56}Fe -depleted $\text{Fe(II)}_{\text{aq}}$ pool in the Archean was required in order to produce depleted pyrite raised the following question: In what sediment is the mass-balancing ^{56}Fe -enriched Fe reservoir recorded (7, 22)? Although BIFs also display some negative $\delta^{56}\text{Fe}$, their mean isotopic composition is ~ 0 to -0.5‰ (Fig. 3) (7). Similarly, even though Archean pyrites display $\delta^{56}\text{Fe}_{\text{pyrite}}$ values down to -3.5‰ , the isotopic distribution clusters between -1.2 and -2.2‰ (Fig. 3). If the pyrite-forming mechanism itself is responsible for the observed compositions, Archean pyrite signatures indicate that the Fe isotope composition of the $\text{Fe(II)}_{\text{aq}}$ reservoir was $\sim 0\text{‰}$ and that the degree of Fe(II) utilization was low. In the Archean environment where $\text{Fe(II)}_{\text{aq}}$ with $\delta^{56}\text{Fe} \sim 0\text{‰}$ is continually injected into the system by hydrothermal vents, small amounts of Fe removal as pyrite would not dramatically change the isotopic composition of the remaining $\text{Fe(II)}_{\text{aq}}$ pool, and we propose that there is no need to invoke the production of a large ^{56}Fe -depleted $\text{Fe(II)}_{\text{aq}}$ pool before pyrite formation. Certainly, both DIR and the removal of $\text{Fe(II)}_{\text{aq}}$ as BIFs in ferruginous regions would promote the production of ^{56}Fe -depleted fluids, enabling the formation of light sedimentary pyrite. However the signatures typically observed in sedimentary pyrite are not diagnostic for DIR. We suggest that low $\delta^{56}\text{Fe}$ signatures in Archean sedimentary pyrite indicate that the portion of Fe removed as pyrite was relatively small with respect to the portion of Fe remaining in the Fe(II) pool (23), which should be tested in geological studies against the degree of pyritization (24). We note that a corre-

lation between Fe and S isotope compositions might also be obtained by varying the degree of Fe and S utilization as pyrite.

The rise of the oxygen into the atmosphere would have stimulated bacterial sulfate reduction through the increase sulfate fluxes because of oxidative continental weathering (25). Persistence of euxinic regions throughout the Proterozoic would have enhanced $\text{Fe(II)}_{\text{aq}}$ removal as sulfides, narrowing down the range of $\delta^{56}\text{Fe}_{\text{pyrite}}$. In the Proterozoic oceans, strongly stratified with oxic shallow waters, euxinic mid-depth proximal regions, and deep, distal ferruginous waters (24, 26), both pyritic black shales and BIFs or their iron oxide precipitate precursors sequestered $\text{Fe(II)}_{\text{aq}}$, and BIFs deposition ended in the late Proterozoic. In these oceans in which $\text{Fe(II)}_{\text{aq}}$ is extensively utilized as pyrite or oxides, $\delta^{56}\text{Fe}_{\text{pyrite}}$ would progressively reflect the values of the $\text{Fe(II)}_{\text{aq}}$ source. The 2.3- to ~ 1.8 -billion-years-ago (Ga) period, for which $\delta^{56}\text{Fe}_{\text{pyrite}}$ displays values up to $\sim +1.2\text{‰}$, may suggest major $\text{Fe(II)}_{\text{aq}}$ source changes in the stratified oceans (11). Where ^{56}Fe -enriched Fe(III) -bearing (hydr)oxides become a dominant $\text{Fe(II)}_{\text{aq}}$ source, abiotic dissolution of Fe(III) (hydr)oxides by H_2S produces ^{56}Fe -enriched $\text{Fe(II)}_{\text{aq}}$ (27), and the isotopic composition of the source would be ^{56}Fe -enriched. Dissolution of siderite would also contribute to the production of ^{56}Fe -enriched $\text{Fe(II)}_{\text{aq}}$ (28). Close temporal and spatial association of ^{56}Fe -enriched pyrite with BIFs (2) supports the idea that pyrite formed through the H_2S dissolution of BIF minerals. Note that theoretical computations predict that at equilibrium pyrite is a heavy phase (4). Under normal sedimentary conditions, it is unlikely that pyrite compositions reflect equilibrium because of the extremely low solubility of pyrite, but higher temperature and late diagenetic

effects could favor isotope exchange toward equilibrium.

The end of the Proterozoic is characterized by the decrease of $\text{Fe(II)}_{\text{aq}}$ in oceans and the spatial limitation of anoxic and ferruginous basins. In this context, DIR and H_2S dissolution of Fe(III) (oxy)hydroxides become the major $\text{Fe(II)}_{\text{aq}}$ source rather than hydrothermal $\text{Fe(II)}_{\text{aq}}$ inputs. The implication is that, where $[\text{S(-II)}]$ is not a limiting factor for pyrite formation, pyrite should display $\delta^{56}\text{Fe}_{\text{pyrite}}$ values approaching those of seawater $\text{Fe(II)}_{\text{aq}}$. $\delta^{56}\text{Fe}_{\text{pyrite}}$ data varying from ~ -0.4 to $\sim -1.2\text{‰}$ in modern anoxic sediments (3, 29) are consistent with the idea that $\delta^{56}\text{Fe}_{\text{pyrite}}$ signatures reflect the degree of Fe utilization as pyrite. In modern sediments, where DIR can be extensive (3), pore water $\text{Fe(II)}_{\text{aq}}$ is ^{56}Fe -depleted. However, fractionation factors cannot be directly calculated from the measured Fe isotopic compositions of the natural phases, because $\text{Fe(II)}_{\text{aq}}$ can remain dissolved for large durations and there is no reason why coexisting $\text{Fe}^{2+}_{\text{aq}}$, FeS_m and pyrite within a sediment should be cogenetic (15). The production of ^{56}Fe -depleted pore water $\text{Fe(II)}_{\text{aq}}$ is not restricted to DIR but also to Fe partial removal as ^{56}Fe -enriched (hydr)oxides and Fe adsorption onto Fe (hydr)oxides (30). In some modern sediments where polysulfide becomes an important $\text{S(-II)}_{\text{aq}}$ species, the pyrite-forming mechanism may be different to those evaluated here and therefore may be accompanied by a different fractionation.

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Supporting Online Material

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Materials and Methods

Fig. S1

Table S1

References (31–41)

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Lévy Walks Evolve Through Interaction Between Movement and Environmental Complexity

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Ecological theory predicts that animal movement is shaped by its efficiency of resource acquisition. Focusing solely on efficiency, however, ignores the fact that animal activity can affect resource availability and distribution. Here, we show that feedback between individual behavior and environmental complexity can explain movement strategies in mussels. Specifically, experiments show that mussels use a Lévy walk during the formation of spatially patterned beds, and models reveal that this Lévy movement accelerates pattern formation. The emergent patterning in mussel beds, in turn, improves individual fitness. These results suggest that Lévy walks evolved as a result of the selective advantage conferred by autonomously generated, emergent spatial patterns in mussel beds. Our results emphasize that an interaction between individual selection and habitat complexity shapes animal movement in natural systems.

Animals must face the daunting complexity of the natural world when searching for food, shelter, and other resources crucial for survival. To cope with the challenge to maximize the probability of resource encounters, many organisms adopt specialized search strategies (1, 2) that can be described by random walks. Brownian and Lévy walks are prominent examples of random walk strategies where both the direction and step length of the constituent moves are drawn from a probability distribution

(1–4). These movement patterns differ in the distribution of step lengths, which are derived from an exponential distribution in the case of Brownian motion, but follow a power-law distribution in case of Lévy motion (4–7), where many short steps are occasionally alternated with a long step. Model simulations have shown that a Lévy walk provides faster dispersal (2, 3), more newly visited sites (1, 2), and less intraspecific competition than Brownian walks (4); it is therefore considered the most efficient random search strategy in resource-limited environments where food occurs patchily at locations unknown to the

searcher (1–3) and, most importantly, where the resource distribution is largely unaffected by the activities of the searching animal (8, 9). Although shown to be optimal for only these specific conditions, Lévy walks are broadly found in nature (1, 10–12), suggesting that they are adaptive over a wider range of conditions. To explain this wide occurrence, we hypothesize that organisms themselves affect the availability and spatial distribution of the resources upon which they depend (13). Consequently, the movement strategies of organisms can shape the environment.

On intertidal flats, the distribution of regularly spaced clumps of mussels (*Mytilus edulis*) results from the interaction between local mussel density and the crawling movement of young mussels (5, 14, 15). In particular, pattern formation in mussel beds is attributable to two opposing mechanisms: cooperation and competition (16). By moving into cooperative aggregations, mussels increase their local density, which decreases wave stress and predation risk. Conversely, competition for algae, which occurs on a larger spatial scale than facilitation, prevents the formation of larger clumps by limiting the number of mussels within a long range. The interaction of local facilitation and long-range competition results in the emergence of a patchy distribution of individuals, which simultaneously reduces risk and minimizes competition for algae (15). Hence, in this system, the distribution of suitable settling locations, an important resource for mussels, is determined by the existing distribution of mussels, which develops in response to the movement of its comprising individuals. Here, we investigate

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Table 1. Goodness-of-fit (G), AIC weights, adjusted R^2 , and Lévy exponents for three classes of movement strategies. The observed step length distribution is best explained by a Lévy walk or a truncated Lévy walk, with Lévy exponents close to 2.

	G	AIC weights	Adjusted R^2	Lévy exponent
Truncated Lévy walk	22.45	0.443	0.997	2.01
Lévy walk	47.22	0.428	0.997	2.06
Brownian walk	−190.09	0.129	0.837	—

whether the interplay between movement strategy and habitat complexity results in the emergence of Lévy walks in these self-organizing mussel beds.

We first tested the hypothesis that mussel movement is described by a Lévy walk (or a truncated Lévy walk) against alternative models reported in the literature, namely, a Brownian walk and a composite Brownian walk (17–19). We observed the movements of 50 mussels during the process of pattern formation and of 12 mussels in solitary experiments in mesocosm tanks. Step lengths were estimated by the distance between two subsequent reorientation events (5). The resulting step length distribution was compared with the family of power-law distributions, $P(l) = Cl^{-\mu}$, where $P(l)$ is the probability of a step of length l and C is a constant ensuring that the total probability equals 1. The exponent μ defines the shape of the distribution and therefore determines the resulting movement strategy. If $1 < \mu < 3$, the movement pattern corresponds to a Lévy walk. When μ approaches 1, the movement is approximately ballistic, while it is approximately Brownian when μ approaches 3 (and for $\mu > 3$) (2, 5, 20) (fig. S2.2). The Lévy walks found in nature typically have an exponent μ of ~ 2 (1, 10–12).

Our results show that mussels use a Lévy walk during the process of pattern formation. On the basis of maximum-likelihood estimation and the derived goodness-of-fit (G), Akaike information criterion (AIC), and the fraction of variance explained by the model (R^2), we found that Lévy walk and truncated Lévy walk distributions, both with $\mu \approx 2$, provided the best fit to the data over a range of at least two orders of magnitude (5) (Table 1, Fig. 1, and table S3.1). A possible alternative explanation is that mussel movement follows a composite Brownian walk, where movement speeds are adjusted to local environmental conditions (17–21). Such a strategy can have a step length distribution similar to that of a Lévy walk and is therefore often overlooked. However, when mussel movements were grouped by local mussel density (the density of mussels within a radius of 3.3 cm) and long-range density (the density of mussels within a radius of 22.5 cm), step length distributions did not differ between the density categories, and mussels were found to perform a Lévy walk with $\mu \approx 2$, irrespective of the local and long-range density (5) (table S3.2). Hence, we reject the hypotheses of Brownian walk and composite Brownian walk and conclude that mussel movement is best described by a Lévy walk.

To examine why mussels adopt a Lévy walk, we investigated the effect of movement strategy on the rate of pattern formation by designing an individual-based model (5). In this model, patterns arise by the mussels' decisions to stay at a location or move away from it. We used experimental data from a previous study to estimate the parameters of this stop-or-move behavior (5, 15) (fig. S2.2). Although step length distributions are

unaffected by mussel density, we found that the probability that a mussel moves decreases with short-range density (the density of mussels within a radius of 3.3 cm) and increases with long-range density (the density of mussels within a radius of 22.5 cm). On the basis of these parameters, simulated mussels stay in places where they can aggregate with direct neighbors, but move away from crowded locations where food becomes limiting. If a simulated mussel moves, the movement distance is randomly drawn from the power-law distribution that corresponds to its movement

strategy. For a range of movement strategies ($1 < \mu \leq 3$), we observed the distance traveled until a pattern has formed. Operationally, we say that a pattern has formed when the density of simulated mussels within 3.3-cm distance is on average 1.5 times as large as the density of mussels within 22.5-cm distance of an individual. Assuming that the movement speed is constant, the rate of pattern formation for each movement strategy is proportional to the inverse of the average distance traversed by the mussels until a pattern has formed (5).

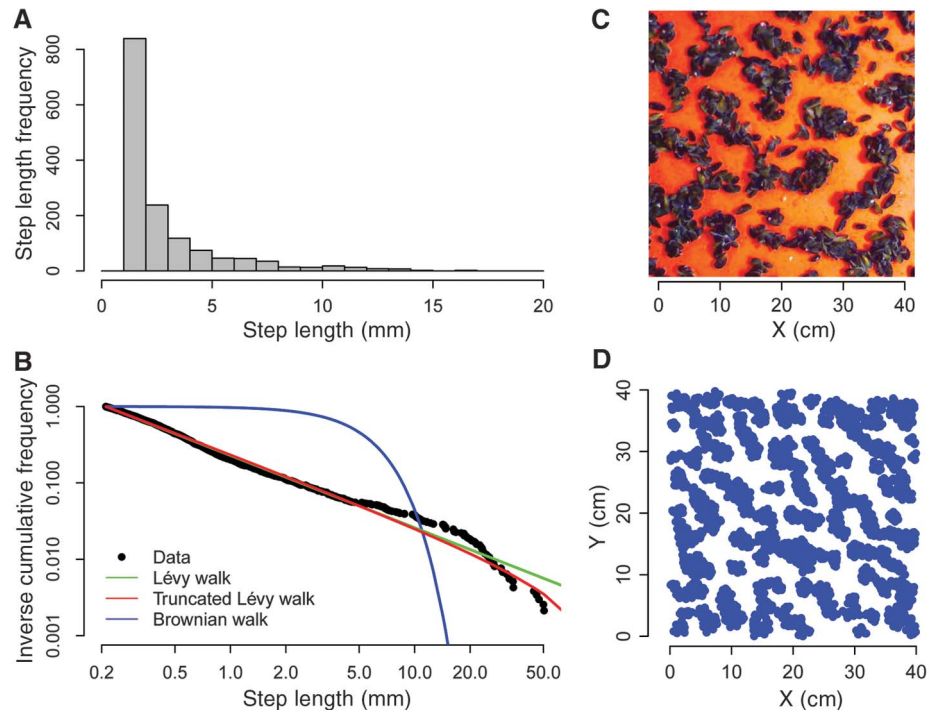


Fig. 1. Experimental and model results showing that mussel movement, which is best described by a Lévy walk, generates patterns in mussel beds. (A) Frequency distribution of step lengths of all solitary mussels (12 mussels, 12,401 steps). (B) Inverse cumulative frequency distribution of the step lengths. (C) Pattern formation in an experimental mussel bed. (D) Pattern generated with our individual-based model.

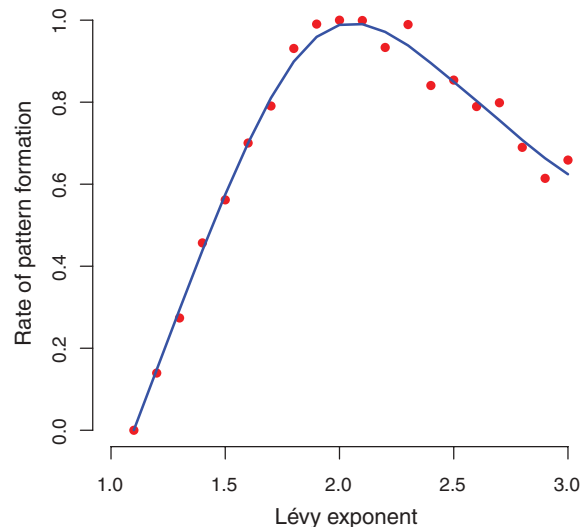


Fig. 2. The rate of pattern formation for various movement strategies. Because we assume that movement speed is constant, we can calculate the rate of patterning as the normalized inverse of the distance traversed until a pattern is formed. A Lévy walk with exponent $\mu \approx 2$ minimizes the time needed to form a pattern.

Simulations reveal that movement strategies differ strongly in terms of the rate at which they create patterns (Fig. 2). A Lévy walk with exponent $\mu \approx 2$ generated a spatially heterogeneous pattern more rapidly than did either ballistic movement ($\mu \rightarrow 1$) or a Brownian walk ($\mu \rightarrow 3$). Specifically, the large steps associated with a small value of μ prevented quick formation of tight clusters, whereas a larger value of μ required many small steps to create clustering. A Lévy walk with $\mu \approx 2$ seems to be the optimal trade-off between finding dispersed conspecifics and maintaining high local densities, thereby maximizing the rate of pattern development. Hence, our simulation results suggest that a Lévy strategy with $\mu \approx 2$ is optimal for pattern formation.

Because pattern formation both improves mussel survival and decreases competition between mussels (14), the movement strategy of individual mussels is likely to be an important determinant of fitness. However, strategies that lead to a desirable outcome at the population level are often not evolutionarily stable, as they can be exploited by free-riding strategies (22). To determine the long-term outcome of selection acting on mussels differing in movement strategy (i.e., their exponent μ), we created a pairwise invasibility plot (PIP, Fig. 3) by performing an evolutionary invasibility analysis (5, 23, 24). The values along the x axis of the PIP represent a broad range of hypothetical resident populations, each with a particular movement strategy characterized by an exponent μ_{res} . The y axis represents the exponents μ_{mut} of potential mutant strategies. The colors indicate whether a mutant strategy μ_{mut} can successfully invade a resident strategy μ_{res} —i.e., whether mutant individuals have a higher fitness than resident individuals in the environment created by the resident population. Intersections between the lines separating the colored areas indicate the presence of an evolutionary attractor, thus predicting the outcome of selection on mussel movement strategies.

Fitness was given by the product of mussel survival (which is proportional to short-range mussel density) and fecundity (which is inversely proportional to long-range mussel density and the energy invested in movement) (5).

The PIP reveals that a Lévy walk with $\mu \approx 2$ is the unique evolutionary attractor of the system (Fig. 3) (23, 24). Specifically, a succession of invasion events will lead to the establishment of a resident population with $\mu \approx 2$, and a resident population with $\mu \approx 2$ cannot be invaded by any other movement strategy. We conclude that the Lévy walk strategy observed in our experiments (Fig. 1) not only has a high patterning efficiency (Fig. 2) but is also an evolutionarily stable strategy (Fig. 3).

Our study demonstrates an evolutionary feedback between individual movement behavior and higher-level complexity and could explain the evolution of Lévy walks in mussel beds. Rather than being a direct adaptation to an externally determined environment, Lévy movement in our study was found to result from feedback between animal behavior and mussel-generated environmental complexity. In essence, a Lévy walk with $\mu \approx 2$ creates a spatial environment in which just this movement strategy can flourish.

Although our study addresses a specific system, the assumption that search strategies can evolve through feedback between animal movement and environmental heterogeneity may be broadly applicable. Such feedbacks may exist not only in the search for conspecifics (as seen here in mussels) but also in the search for resources shared with conspecifics, because resource patterns reflect the movement patterns of their consumers. This applies, for instance, to the interaction between herbivores and vegetation, which shapes grasslands globally (25). Additionally, feedback between movement strategy and habitat complexity may arise when the spatial distribution of a particular species depends on interactions with a searching organism [as in predator-prey relationships or animal-mediated seed dispersal (26)].

We conclude that the interaction between animal movement and habitat complexity is a key component in understanding the evolution of animal movement strategies.

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Supporting Online Material

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Materials and Methods

Figs. S2.1 to S2.3

Tables S3.1 and S3.2

References and Notes

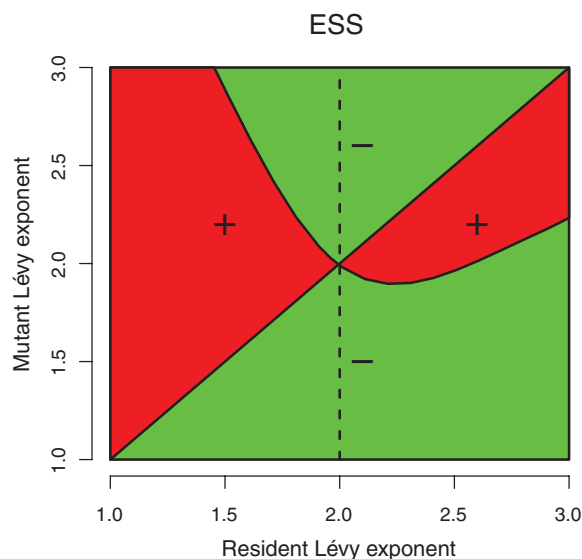
Movie S1

Matlab code for individual-based model of mussel movement (The MathWorks, Inc.)

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10.1126/science.1201187

Fig. 3. Pairwise invasibility plot (PIP) indicating that the movement strategy evolves toward a Lévy walk with $\mu \approx 2$. For a range of resident (x axis) and mutant (y axis) movement strategies, the PIP indicates whether a mutant has a higher (red) or a lower (green) fitness than the resident and, hence, whether a mutant can invade the resident population (23). Here, the PIP shows that a Lévy walk with $\mu \approx 2$ is the sole evolutionarily stable strategy (ESS).



Gametogenesis Eliminates Age-Induced Cellular Damage and Resets Life Span in Yeast

Elçin Ünal,¹ Benyam Kinde,^{2*} Angelika Amon^{1†}

Eukaryotic organisms age, yet detrimental age-associated traits are not passed on to progeny. How life span is reset from one generation to the next is not known. We show that in budding yeast resetting of life span occurs during gametogenesis. Gametes (spores) generated by aged cells show the same replicative potential as gametes generated by young cells. Age-associated damage is no longer detectable in mature gametes. Furthermore, transient induction of a transcription factor essential for later stages of gametogenesis extends the replicative life span of aged cells. Our results indicate that gamete formation brings about rejuvenation by eliminating age-induced cellular damage.

Most, if not all, eukaryotic organisms age; however, the age-induced changes are not transmitted to the progeny. How life span is reset from one generation to the next is not known. We wished to test the hypothesis that resetting of life span occurs during gametogenesis. In budding yeast, gamete formation (sporulation) requires meiosis and includes the generation of new membrane compartments, protein and organelle degradation, and synthesis of a resistant spore wall (1). To determine whether gamete formation causes rejuvenation, we asked whether spores derived from aged cells have reset their life span and are young or whether they inherit the progenitor's age and remain old. We isolated replicatively aged cells on the basis of biotin labeling of mother cells (2) and induced them to sporulate in the same flask as young cells (fig. S1). Upon sporulation, tetrads were dissected and the replicative life span (RLS) of each spore was measured. We found that the life spans of the spores derived from young and aged cells were indistinguishable in two *Saccharomyces cerevisiae* strain backgrounds: W303, in which sporulation efficiency decreases with age (3, 4) (Fig. 1B), and A364a, in which sporulation efficiency remains high despite aging (4) (fig. S2). In contrast, aged cells obtained by the same procedure but not induced to sporulate die rapidly (Fig. 1A). The RLS of the four spores from a tetrad produced from young and aged cells is the same; no statistically significant differences are observed (4) (Fig. 1C). This is in contrast to mitosis, where age is asymmetrically inherited between the mother cell and the bud, culminating in the production of a young daughter and an old mother cell (5). Thus, sporulation resets RLS.

We next asked how sporulation affects age-dependent cellular changes, such as increased levels of protein aggregation (6), aberrant nucleolar structures, and increased levels of extra-chromosomal ribosomal DNA (rDNA) circles (ERCs) (7, 8). In budding yeast, protein aggregates associate with Hsp104 and form foci in replicatively aged cells, which can be visualized by Hsp104 joined to enhanced green fluorescent protein (Hsp104-eGFP) (6) (fig. S3, A to D). During vegetative growth, Hsp104-eGFP foci are distributed asymmetrically between the mother and

daughter cell; less than 10% of young cells displayed foci, but Hsp104-eGFP foci began to accumulate by generation eight in mother cells and were present in 85% of aged cells (4, 6, 9) (fig. S3D). Throughout sporulation, most aged cells contained Hsp104-eGFP foci [mononucleates (95%), binucleates (90%), and tetranucleates (86%)], but the foci were essentially absent in mature tetrads (3%) (Fig. 2, A and B), which suggests that age-associated protein aggregates are cleared during sporulation. The polarisome is required for the asymmetric distribution of Hsp104-eGFP foci during mitosis (9). Deleting the genes encoding the polarisome components Bud6 or Spa2 did not interfere with aggregate elimination during sporulation (fig. S3, E and F). Proteasome function also appeared dispensable for aggregate clearance. Treatment of cells with the proteasome inhibitor MG132 after the second division prevented neither sporulation nor the clearance of Hsp104 aggregates (10) (fig. S3, G and H). In contrast, treatment of cells with the autophagy inhibitor chloroquine prevented sporulation, and aggregates persisted (11) (fig. S3, G and H), which suggests that an autophagy-dependent process and/or spore formation are required for aggregate clearance.

Aged cells are also defective in rDNA metabolism, displaying fragmented nucleoli and forming extra chromosomal rDNA circles (ERCs) (7, 8).

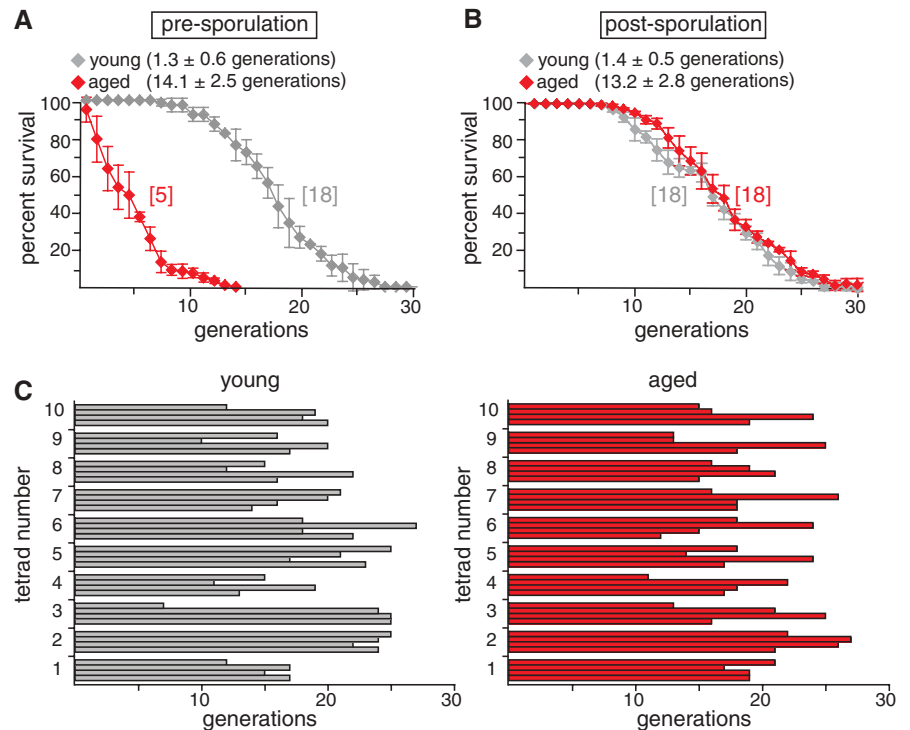


Fig. 1. Gametogenesis resets RLS. The average number of cell divisions of the starting cell population is indicated in the keys. The median life span is written next to each curve. Error bars denote standard deviation. (A) RLSs of young and aged wild-type A702 cells, directly after sorting, before sporulation. (B) Postsporulation RLSs of spores from young and aged A702 cells. (C) Age distribution of spores from A702 in individual tetrads from young and aged progenitors, $n = 10$. The life span of spores from each tetrad is compared with the mean life span of young spores to obtain a P value. The average P value from 10 tetrads is 0.303 for young and 0.642 for aged spores (t test), which indicates no statistically significant difference in replicative age among spores from a given tetrad.

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ERCs decreased considerably during sporulation in aged cells, reaching levels similar to those of young cells (Fig. 2C and fig. S4). In aged cells, the rDNA structure also underwent dramatic changes as judged by the localization of Fob1-GFP, a nucleolar protein that binds to the rDNA (12). In 60% of aged cells, the Fob1-GFP appeared enlarged and discontinuous, which probably reflects

rDNA condensation defects and nucleolar fragmentation, respectively. After aged cells sporulate, more than 90% of the tetrads contained a single Fob1-GFP focus per spore and displayed a morphology indistinguishable from that of young cells (Fig. 2D). Together, our results demonstrate that gamete formation eliminates age-induced protein aggregation and nucleolar aberrations.

To determine which aspects of gametogenesis are necessary for RLS resetting, we deleted two transcription factors that trigger different stages of sporulation and asked whether RLS was reset. Such studies are possible because in budding yeast, sporulating cells can resume vegetative growth (return to growth) (4), provided that the sporulation-inducing cue, nutrient deprivation,

Fig. 2. Sporulation eliminates age-induced cellular damage. **(A)** Analysis of Hsp104-eGFP aggregates in aged sporulating A25825 cells. **(B)** Quantification of Hsp104-eGFP foci in young (1.3 ± 0.6 generations) and aged (6.8 ± 1.3 generations) A25825 cells before meiosis I (mononuc), after meiosis I (binuc), after meiosis II (tetranuc), and in tetrads. **(C)** rDNA and ERCs in young (1.3 ± 0.5 generations) and aged (15 ± 2.7 generations) A26370 cells. **(D)** Nucleolar morphology in young (1.4 ± 0.6 generations) and aged (14.2 ± 6.1 generations) A26271 cells.

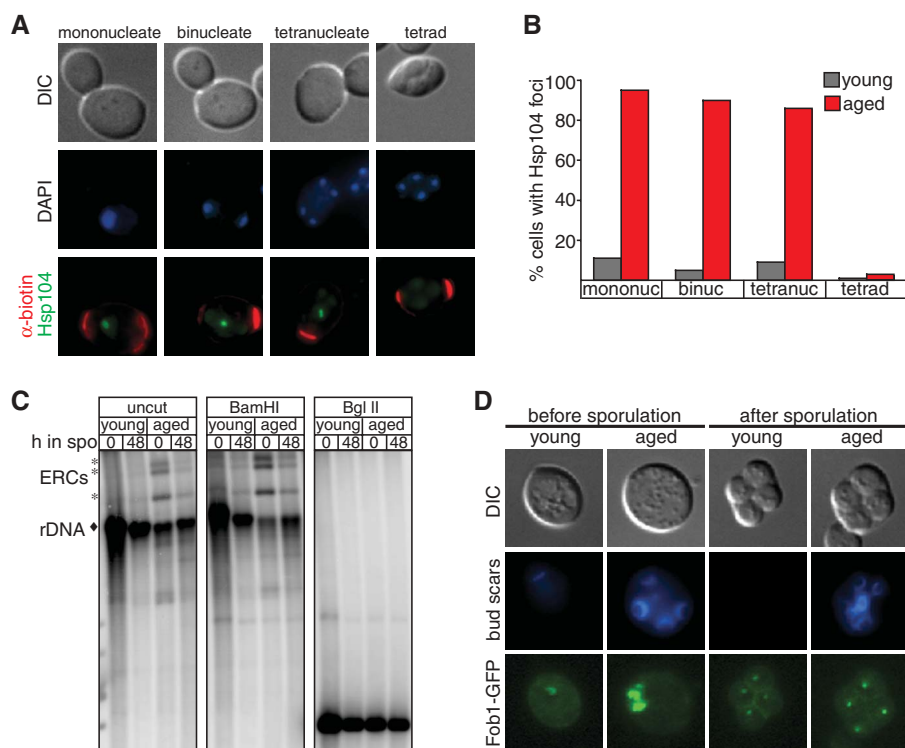
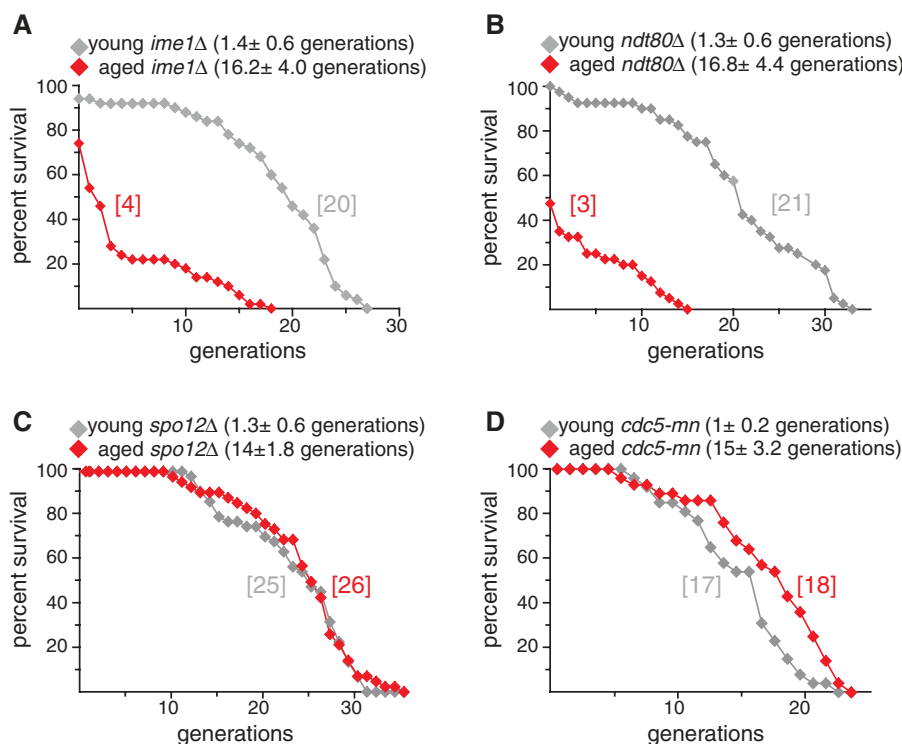


Fig. 3. *IME1* and *NDT80*, but not the meiotic nuclear divisions, are required for life-span resetting. **(A)** RLSs of young and aged A23998 (*ime1* Δ) cells. **(B)** RLSs of young and aged A24074 (*ndt80* Δ) cells. The median life span of the aged cells is 0; therefore, the average is shown. **(C)** The life spans of young and aged A27377 (*spo12* Δ) spores. **(D)** The life spans of young and aged A24142 (*cdc5-mn*) spores.



is withdrawn. We first analyzed the RLSs of young (1.4 ± 0.6 generations) and aged (16.2 ± 4 generations) cells from a strain that lacked *Ime1*. Without *Ime1*, yeast cells are unable to initiate sporulation but still sense nutrient deprivation (13). To ensure that only cells that responded to the sporulation-inducing cues were included in the analysis, we used a *pIME1:mCherry* reporter construct (fig. S5A) (14). Aged *ime1Δ* cells lost viability rapidly, with a median life span of four generations (Fig. 3A). Similar results were obtained with wild-type cells that had responded to sporulation cues as judged by *pIME1:mCherry* expression but had not yet entered the sporulation program (fig. S5B). Thus, the initiation of sporulation driven by *IME1* is required to reset RLS. Furthermore, nutrient deprivation and other sporulation signals are insufficient to promote RLS resetting.

In the absence of the transcription factor Ndt80, yeast cells complete premeiotic DNA replication, initiate recombination, and arrest in pachytene (15). We used Zip1-GFP to identify the *ndt80Δ* cells arrested in pachytene (fig. S5C) (16) and found that young cells (1.3 ± 0.6 generations) resumed vegetative growth and divided an average of 21.6 ± 7.9 times (median RLS = 21). In contrast, almost 50% of the aged cells (16.8 ± 4.4 generations) lost viability within the first mitotic division. The remaining cells underwent significantly fewer divisions compared with young

cells (3.4 ± 4.9 generations) (Fig. 3B). We conclude that *NDT80*-induced processes are necessary for RLS resetting, and that the events before *NDT80* function, such as premeiotic DNA replication and recombination, are insufficient to promote rejuvenation.

Progression through sporulation up to pachytene is not sufficient for resetting of RLS. Thus, later stages of sporulation, such as the meiotic nuclear divisions and/or spore formation, must be required. To determine whether both meiotic divisions are necessary for RLS resetting, we deleted *SPO12*. The resulting *spo12Δ* cells undergo a single nuclear division and form two diploid spores (17). We found that young (1.3 ± 0.6 generations) and aged (14 ± 1.8 generations) *spo12Δ* cells had indistinguishable RLSs, which suggests that two consecutive meiotic divisions are not a prerequisite for rejuvenation (Fig. 3C). The life span of spores within individual two-spored asci is very similar (fig. S6) (for the young, $P = 0.15$; for the aged, $P = 0.29$, $n = 30$, Wilcoxon signed-rank test). To test if resetting of RLS can occur in the absence of any nuclear divisions, we inactivated the pololike kinase Cdc5 during sporulation (*cdc5-mn*) (18). Cells lacking Cdc5 do not undergo any meiotic divisions and form single spores. Like *spo12Δ* spores, *cdc5-mn* spores obtained from aged cells regained their replicative potential (Fig. 3D). We conclude that the meiotic divisions per se are dispensable for RLS resetting and note that our

findings exclude a model where halving of the genome or diluting aging factors brings about the resetting of RLS (4).

NDT80-regulated genes that mediate spore formation could be required for rejuvenation. As *NDT80* expression is sufficient to induce the expression of mid- and late-sporulation genes in vegetative cells (19, 20), we determined whether Ndt80 could extend the life span of vegetative cells. We expressed *NDT80* from the *GAL1-10* promoter (*GAL-NDT80*), whose expression can be regulated by a Gal4–estrogen receptor fusion (Gal4.ER) (21, 22) (fig. S7, A and B). Expression of *NDT80* significantly extended the life span of mitotic cells (fig. S7, C and D) ($P < 0.0001$, Z score = 4, Mann-Whitney test). To test whether induction of *NDT80* in replicatively aged cells also extends life span, we transiently induced Ndt80 with β -estradiol in young and aged cells and followed their RLSs in the absence of β -estradiol. Aged cells that transiently expressed *NDT80* lived significantly longer than aged cells treated in the same manner but lacking the *GAL-NDT80* fusion (Fig. 4A) ($P < 0.0001$, Z score = 8.23, Mann-Whitney test). Transient expression of *NDT80* even led to an extension of life span in young cells (Fig. 4A) ($P < 0.0001$, Z score = 4.85, Mann-Whitney test). Similar results were obtained in experiments comparing cell divisions in liquid culture, excluding the possibility that transient expression of *NDT80* extends life span because

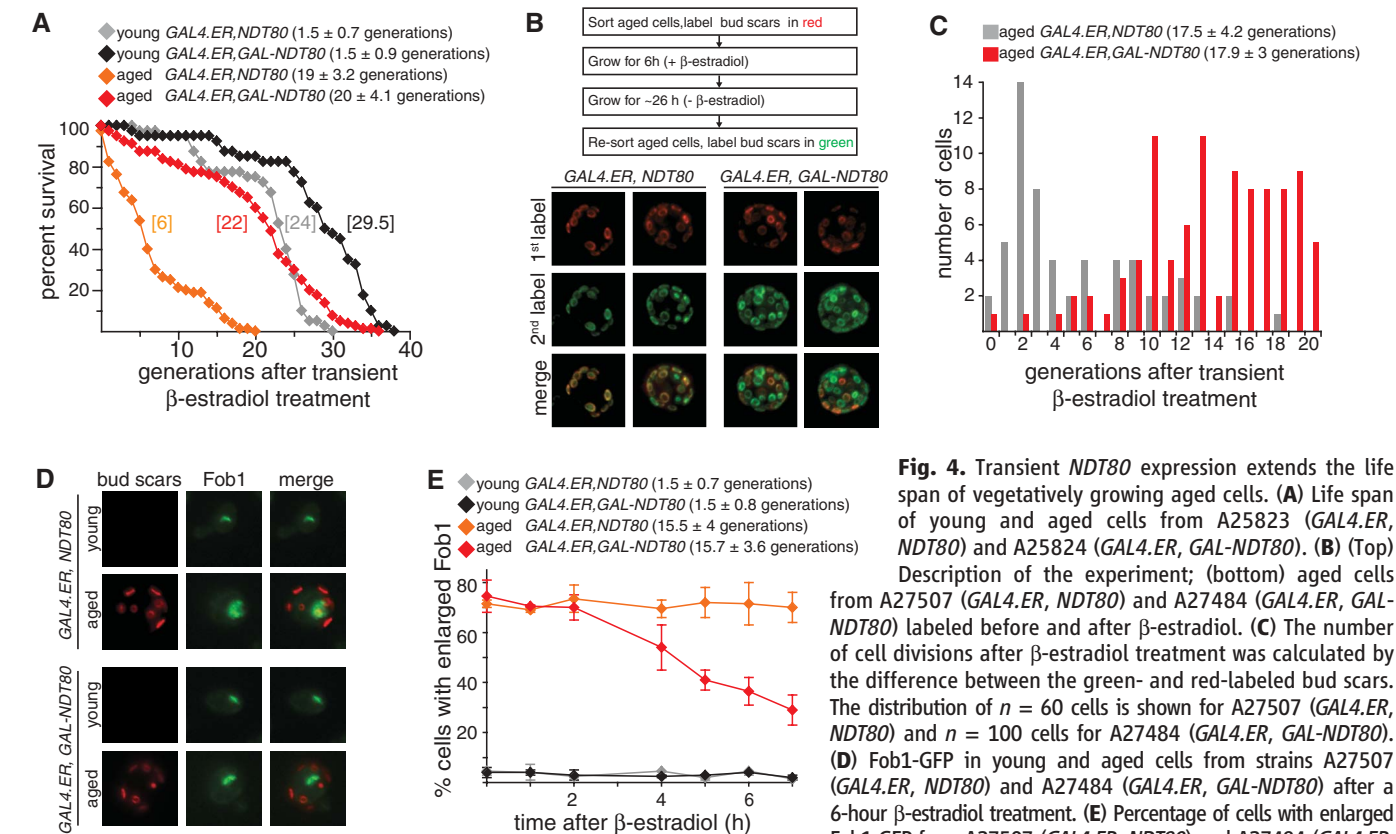


Fig. 4. Transient *NDT80* expression extends the life span of vegetatively growing aged cells. (A) Life span of young and aged cells from A25823 (*GAL4.ER, NDT80*) and A25824 (*GAL4.ER, GAL-NDT80*). (B) (Top) Description of the experiment; (bottom) aged cells from A27507 (*GAL4.ER, NDT80*) and A27484 (*GAL4.ER, GAL-NDT80*) labeled before and after β -estradiol. (C) The number of cell divisions after β -estradiol treatment was calculated by the difference between the green- and red-labeled bud scars. The distribution of $n = 60$ cells is shown for A27507 (*GAL4.ER, NDT80*) and $n = 100$ cells for A27484 (*GAL4.ER, GAL-NDT80*). (D) Fob1-GFP in young and aged cells from strains A27507 (*GAL4.ER, NDT80*) and A27484 (*GAL4.ER, GAL-NDT80*) after a 6-hour β -estradiol treatment. (E) Percentage of cells with enlarged Fob1-GFP from A27507 (*GAL4.ER, NDT80*) and A27484 (*GAL4.ER, GAL-NDT80*) following β -estradiol treatment. The average of two independent experiments is shown. 100 to 200 cells were counted for each time point; error bars display the range.

it causes cells to become more resistant to the micromanipulations involved in the pedigree analysis (Fig. 4, B and C). Thus, a transient induction of *NDT80* is sufficient to extend the life span of replicatively aged cells.

To address how transient induction of Ndt80 extends RLS, we monitored age-dependent cellular changes after *NDT80* induction. Neither ERCs nor Hsp104-eGFP aggregates were reduced after *NDT80* induction, although it is possible that they were reduced at later time points (fig. S8, A and B). Furthermore, *NDT80* expression extended life span in the absence of the autophagy gene *ATG1* (fig. S8C). Together these findings suggest that life-span extension can occur in the absence of ERC and Hsp104-aggregate elimination. Although ERCs and Hsp104-eGFP aggregates were not affected by transient *NDT80* expression, nucleolar morphology was. The percentage of aged cells with enlarged nucleolar morphology decreased after *NDT80* induction (Fig. 4, D and E, and fig. S8D). Thus, transient induction of *NDT80* causes a change in nucleolar and/or rDNA structure, which reverts to a state that resembles the morphology of young cells.

We do not yet know whether *NDT80*- and sporulation-induced RLS resetting use the same mechanism(s). The findings that *NDT80* is necessary for life-span extension during sporulation and sufficient for life-span extension during vegetative growth and that nucleolar morphology is altered under both circumstances suggest that at least some processes are shared. Irrespective of the relation between *NDT80*- and sporulation-induced RLS resetting, we note that resetting of RLS provides an opportunity to dissect the molecular causes of aging. For instance, elimination

of Hsp104 aggregates and ERCs seem unlikely to be required for *NDT80*-dependent life-span extension, but changes in nucleolar function and/or structure may be important. Intriguingly, rDNA instability and not ERCs per se appear to cause aging in yeast (23), and budding yeast cells eliminate most of the nucleolar material during spore packaging (24).

It will be interesting to investigate whether our findings extend to other species. In *Caenorhabditis elegans*, a number of longevity mutants exhibit a soma-to-germline transformation that contributes to their enhanced survival (25). In mice, re-introduction of telomerase rescues the age-related phenotypes of telomerase-deficient mice (26), which suggests that age-dependent cellular damage can be repaired. Our studies suggest that a transient induction of the gametogenesis program in somatic cells removes age-dependent cellular damage and extends life span. Determining how gametogenesis causes the resetting of life span will provide insights into the mechanisms of aging and could facilitate the development of strategies for longevity.

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Materials and Methods

SOM Text

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A Cell Cycle Phosphoproteome of the Yeast Centrosome

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Centrosomes organize the bipolar mitotic spindle, and centrosomal defects cause chromosome instability. Protein phosphorylation modulates centrosome function, and we provide a comprehensive map of phosphorylation on intact yeast centrosomes (18 proteins). Mass spectrometry was used to identify 297 phosphorylation sites on centrosomes from different cell cycle stages. We observed different modes of phosphoregulation via specific protein kinases, phosphorylation site clustering, and conserved phosphorylated residues. Mutating all eight cyclin-dependent kinase (Cdk)-directed sites within the core component, Spc42, resulted in lethality and reduced centrosomal assembly. Alternatively, mutation of one conserved Cdk site within γ -tubulin (Tub4-S360D) caused mitotic delay and aberrant anaphase spindle elongation. Our work establishes the extent and complexity of this prominent posttranslational modification in centrosome biology and provides specific examples of phosphorylation control in centrosome function.

Phosphorylation is a reversible posttranslational modification that regulates most cellular processes, including the duplication of centrosomes to form the mitotic spindle, which functions in chromosome segregation. Protein ki-

nases, such as cyclin-dependent kinase Cdk1 (Cdc28), Mps1, and Polo kinase (Cdc5) (1, 2), phosphorylate the centrosome, known in yeast as the spindle pole body (SPB; Fig. 1A). The 18 centrosomal proteins (10 have human homologs;

Figs. 1B and 2) can be organized into five functional subcomplexes (1): the γ -tubulin complex (Tub4, Spc98, and Spc97), which nucleates microtubules; the central core (Nud1, Spc42, Spc29, and Cnm67), which form the organelle's structural foundation and precursor; the linker proteins connecting the core and γ -tubulin complexes; the membrane anchors; and the half-bridge components, where assembly begins. Previous studies examined phosphorylation of these components individually or within whole cell preparations (database S1, column 3). In contrast, we performed

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a comprehensive analysis of phosphorylation on enriched, intact centrosomes.

Centrosomal complexes were isolated from yeast cells by using a modified affinity purification (3) (fig. S1A), and copurifying proteins were analyzed by solution digest and mass spectrometry (MS). Phosphopeptides were enriched with a metal affinity column, processed by liquid chromatog-

raphy tandem mass spectrometry (LC MS/MS) on an LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific, San Jose, CA), and identified with SEQUEST and DTASelect2 programs (4). DeBunker (5) and Ascore (6) programs were used to further validate phosphopeptides and phosphorylation assignments, respectively (database S2). Peptides from all 18 proteins were

identified, with extensive peptide coverage for most proteins (Fig. 2). The centrosomal preparations (fig. S1B) were highly phosphorylated (fig. S1C), as observed by MS analysis (www.yeastrc.org/pdr/pages/front.jsp; search by protein name). In total, 297 phosphorylation events were mapped on 17 of the 18 yeast centrosomal proteins, of which 227 have not been previously reported.

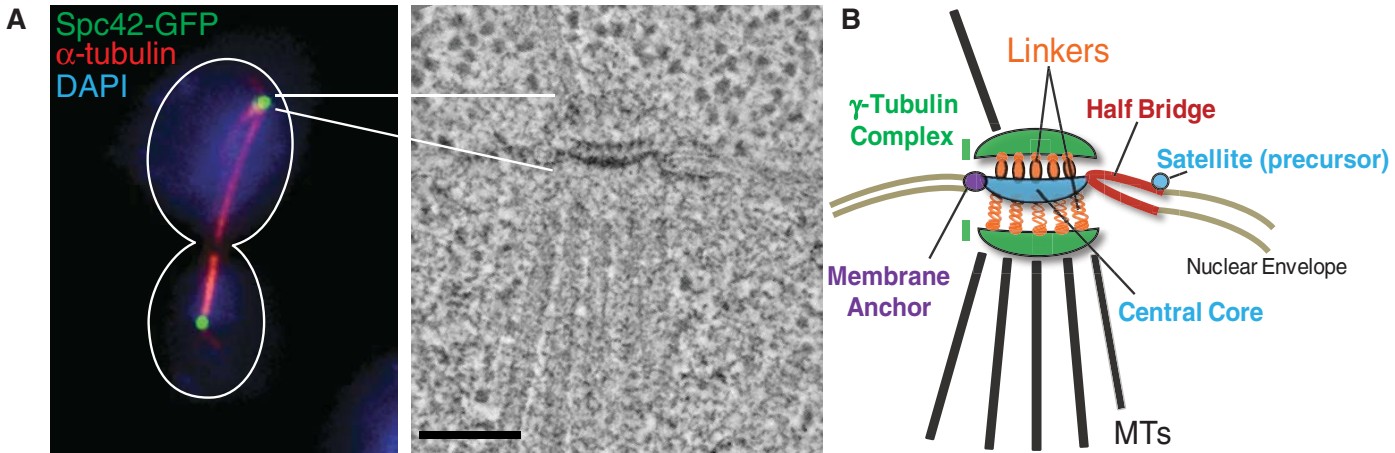


Fig. 1. Yeast centrosomes form the poles of the mitotic spindle and are composed of five subcomplexes. **(A)** Immunofluorescence (left) of a large-budded mitotic yeast cell showing centrosomes marked by Spc42–green fluorescent protein (GFP) (green), microtubules (red), and DNA (blue), and

electron micrograph (right) showing trilaminar ultrastructure. Scale bar indicates 100 nm. [Credit: Eileen O’Toole, University of Colorado, Boulder] **(B)** Schematic of the five major functional centrosome subcomplexes. MT indicates microtubule.

Fig. 2. Phosphoproteomic analysis of enriched *Saccharomyces cerevisiae* centrosomes organized by centrosome complexes. Total sites indicates all phosphorylation sites found within all asynchronous, mitotic, and G1 preparations; numbers in parentheses are ambiguous assignments (databases S1 and S2); S/T(P) sites, potential Cdk1 sites; Y sites, tyrosine phosphorylation sites; Coverage, % of the total protein sequence recovered as peptides from mass spectrometry analyses of all centrosome preparations; and human homologs are indicated if applicable. Check marks indicate proteins that are known in vivo or in vitro substrates of Cdk1, Mps1, or Cdc5 kinases (references in fig. S4); dash entries, not observed; asterisks, homologous domains.

Centrosome Proteins	Total Sites	S/T (P) Sites	Y Sites	Coverage	Cdk Kinase	Mps1 Kinase	Cdc5 Kinase	Human Homologs
γ-Tubulin Complex								
Tub4	8 (1)	1	2	85%	✓	-	-	TUBG1
Spc98	9	2	2	65%	✓	✓	-	TUBGCP3
Spc97	5 (1)	0	1	54%	-	-	-	TUBGCP2
Linkers								
Spc110	31	3	3	96%	✓	✓	-	Kendrin
Spc72	19 (2)	3	0	92%	-	-	✓	TACC*
Cmd1	7 (1)	0	0	91%	-	-	-	Calmodulin
Core and Satellite								
Nud1	52 (2)	5	1	91%	✓	-	✓	Centriolin
Spc42	31 (1)	6	3	96%	✓	✓	-	
Spc29	32 (2)	4	0	100%	✓	✓	-	
Cnm67	22	6	3	100%	✓	-	-	
Half Bridge								
Kar1	7 (1)	2	0	63%	-	-	-	
Sfi1	11	4	0	42%	✓	-	-	Hsfi1
Cdc31	4	0	0	98%	-	✓	-	Centrin3
Mps3	0	0	0	7%	-	-	-	SUN domain*
Membrane Anchor								
Nbp1	27	6	5	90%	✓	-	-	
Bbp1	20	3	2	85%	✓	-	-	
Mps2	11	4	0	75%	✓	-	-	
Ndc1	1	0	0	24%	-	-	-	
TOTAL:	297(11)	49	22					

Among these are 49 potential Cdk1 sites [S/T-P, serine or threonine followed by proline, 5 are confirmed as Cdk sites (7)] and 22 tyrosines (Fig. 2). Combining data from this study (297 sites) and previous studies (29 sites), a total of 326 phosphorylation sites are now identified on the yeast centrosome (database S1).

Because phosphorylation regulates cell cycle events (8), including centrosome duplication and mitotic spindle formation, we explored phosphorylation profile differences between centrosomes in cell cycle-arrested cells versus those growing asynchronously. Cells were arrested at an early step of centrosome duplication in late G1 phase with α -factor treatment or in mitosis after centrosome duplication and separation by depletion of the anaphase-promoting complex (APC) activator Cdc20 (fig. S2A). We detected 54 sites that were phosphorylated only in G1, 110 sites that were phosphorylated only in mitosis, and 68 sites phosphorylated in both phases (Fig. 3, fig. S2B, and database S1). The latter 68 sites are likely to be constitutively phosphorylated (because 61 were also found in asynchronous preparations). Of all the subcomplexes, the central core contained the

largest number of sites (46% of the total, Fig. 2) and also the highest percentage of all shared sites (72%) (fig. S2C). The 29 mitotic sites on Nud1 may affect its subsequent role in recruitment of cell cycle regulatory proteins required for mitotic exit (9). In contrast to the central core, the γ -tubulin complex, linkers, and half-bridge have few constitutive sites and a large number of sites phosphorylated in mitosis.

Analysis of phosphorylated residues that are likely within binding sites or targets of specific kinases showed distinct cell cycle patterns. For example, the majority of sites within Polo (Cdc5) binding motifs (fig. S3A) were observed in mitosis when its activity peaks (10). Cell cycle-specific phosphorylation was also observed in 21 of 22 tyrosine sites. In contrast, over half (27 of 49) of the potential Cdk consensus sites were phosphorylated throughout the cell cycle. The constitutive Cdk phosphorylation in Spc42 appeared to be essential, because mutating the Cdk motifs to nonphosphorylatable residues [8 sites out of 32 total phosphorylation sites in Spc42 (fig. S3B)] was lethal. The lethality may result, in part, from the decrease in Spc42 assembly into the

centrosome (Fig. 4A). Furthermore, phosphorylation of these Cdk sites is critical for overall Spc42 phosphorylation, because phosphate incorporation decreased in the Spc42-8A mutant by 93% compared with the wild type (WT) (Fig. 4B).

Twelve centrosomal proteins are known substrates of Cdk1 or Mps1 (Fig. 2 and fig. S4A). We performed kinase reactions in vitro with either Cdk1 or Mps1 on our centrosome preparations and identified potential centrosomal substrates by in-gel digestion and MS analysis (fig. S4B). We observed kinase specificity on centrosomal substrates by distinct phosphorylation banding patterns, confirmed several substrates, and identified possible new substrates (Spc72 and Cnm67) for Mps1 and Cdk1, respectively.

Clustering of phosphorylation sites is a rare event that creates a charged region, which can affect protein interactions and contribute to structural integrity (11). A study of yeast Cdk phosphorylation showed that a cluster of sites, rather than individual residues, can be evolutionarily conserved (7). Clustering was prominent within our centrosomal phosphoproteome, with 174 of the 297 mapped sites clustered in seven proteins [fig. S5, ≥ 5 sites within 50 residues (4)]. Twenty-nine of the 49 Cdk consensus sites were included within these clustered regions. The importance of phosphorylation site clustering is exemplified by analysis of the N terminus of Spc110, which interacts with Spc97 to stabilize the γ -tubulin complex (12, 13). Mutating even 2 out of the 18 phosphorylation sites in this region (fig. S5) is lethal when combined with *spc97* mutations (14).

Individual residues that are functionally and structurally important are also conserved through evolution (15, 16). We therefore examined fungal orthologs of centrosomal proteins to determine evolutionary constraint values [measured by positional conservation (17, 18)] for the 297 sites and also regional conservation throughout the proteins [fig. S6; see Fig. 5, A and B, for γ -tubulin (Tub4)]. This analysis identified 59 highly constrained sites in 13 proteins ($>80\%$ conserved) and 14 fully conserved residues in 8 proteins (fig. S7, A and B), of which three sites, Tub4-Y445, Spc29-T18, and Spc29-T240 (19), are essential for centrosome function (20–22). Fourteen sites from this study are conserved in human centrosomal proteins (fig. S7C).

The phosphorylated residue, S360 (19), within γ -tubulin (Tub4) (fig. S8A) is fully conserved in fungi (Fig. 5B and fig. S6) and in humans (fig. S8B). γ -Tubulin is part of an evolutionarily conserved complex (γ -tubulin small complex, γ -tuSC) that nucleates microtubules for chromosome segregation. Phosphorylation of γ -tubulin has been shown to promote centrosome duplication and microtubule assembly (20, 23). Tub4-S360 lies within a Cdk motif and is phosphorylated by Cdk1 in vitro (fig. S8, C and D). This site is located within a surface loop available for protein-protein interactions, as viewed in the γ -tubulin crystal structure (Fig. 5C, star). Furthermore, structural analysis using cryo-electron microscopy

Centrosome Proteins	Total Asynch	Unique Asynch	Total G1	Unique G1	Total Mitotic	Unique Mitotic	Shared G1/M
γ-Tubulin Complex							
Tub4	0	0	2	2	6	6	0
Spc98	3	3	3	2	4	3	1
Spc97	0	0	3	3	2	2	0
Linkers							
Spc110	16	6	14	9	16	11	5
Spc72	5	2	2	2	15	15	0
Cmd1	4	4	1	1	2	2	0
Core and Satellite							
Nud1	27	10	13	2	40	29	11
Spc42	25	1	20	3	27	10	17
Spc29	26	8	18	8	16	6	10
Cnm67	16	2	15	4	16	5	11
Half Bridge							
Kar1	6	3	0	0	4	4	0
Sfi1	7	4	2	2	5	5	0
Cdc31	3	2	1	0	2	1	1
Mps3	0	0	0	0	0	0	0
Membrane Anchor							
Nbp1	20	10	12	7	10	5	5
Bbp1	12	4	12	6	10	4	6
Mps2	9	6	4	3	2	1	1
Ndc1	1	0	0	0	1	1	0
TOTAL:	180	65	122	54	178	110	68

Fig. 3. *S. cerevisiae* centrosome phosphorylation is dynamic during the cell cycle and is enriched in mitosis. Total Asynch, all sites found in asynchronous preparations or Unique, only in Asynch; Total G1, all sites found in G1 preparations or Unique in G1 (not in mitotic preparations); Total Mitotic, all sites found in mitotic preparations or Unique in mitotic (not in G1 preparations); Shared G1/M, sites found in mitotic and G1 preparations.

of the yeast γ -tubulin complex places this loop directly between Spc98 and Spc97 (13). Therefore, we mutated S360 to either a nonphosphorylatable alanine (A) or an aspartic/glutamic acid (D or E) to mimic constitutive phosphorylation. The *tub4-S360A* allele did not affect growth; however, *tub4-S360D* and *tub4-S360E* caused growth defects at 25°C and mitotic arrest resulting in cell death upon shift to a higher temperature (37°C) (fig. S9, A to C). Also, *tub4-S360D* was lethal in combination with mutations in *SPC98* (*spc98-2*), by deletion of the spindle checkpoint gene *MAD2*

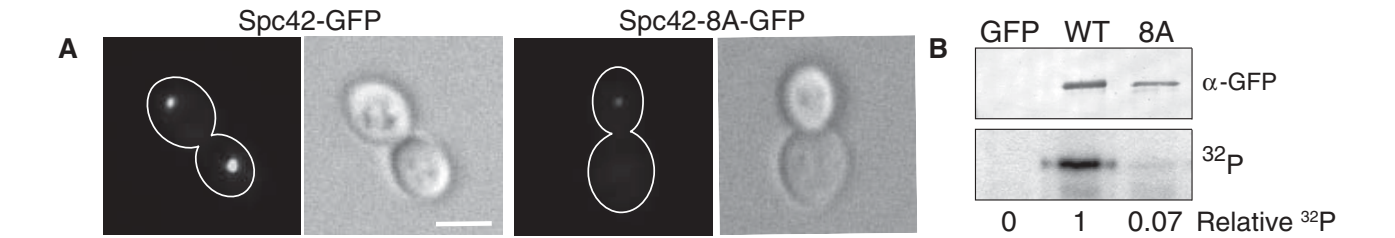


Fig. 4. Effect of mutating all Cdk sites within the core protein, Spc42. (A) Incorporation of Spc42-GFP and Spc42-8A-GFP into the two centrosomes, analyzed by fluorescence microscopy ($n = 200$). Phase microscopy images show large-budded mitotic cells. Scale bar, 5 μ m. (B) Relative 32 P incorporation into GFP, Spc42-GFP (WT), and Spc42-8A-GFP (8A) proteins upon protein induction in yeast cells shown by anti-GFP Western blot (top) and autoradiograph (bottom). Quantified by phosphorimager (below) and normalized to α -GFP signal, $n = 3$.

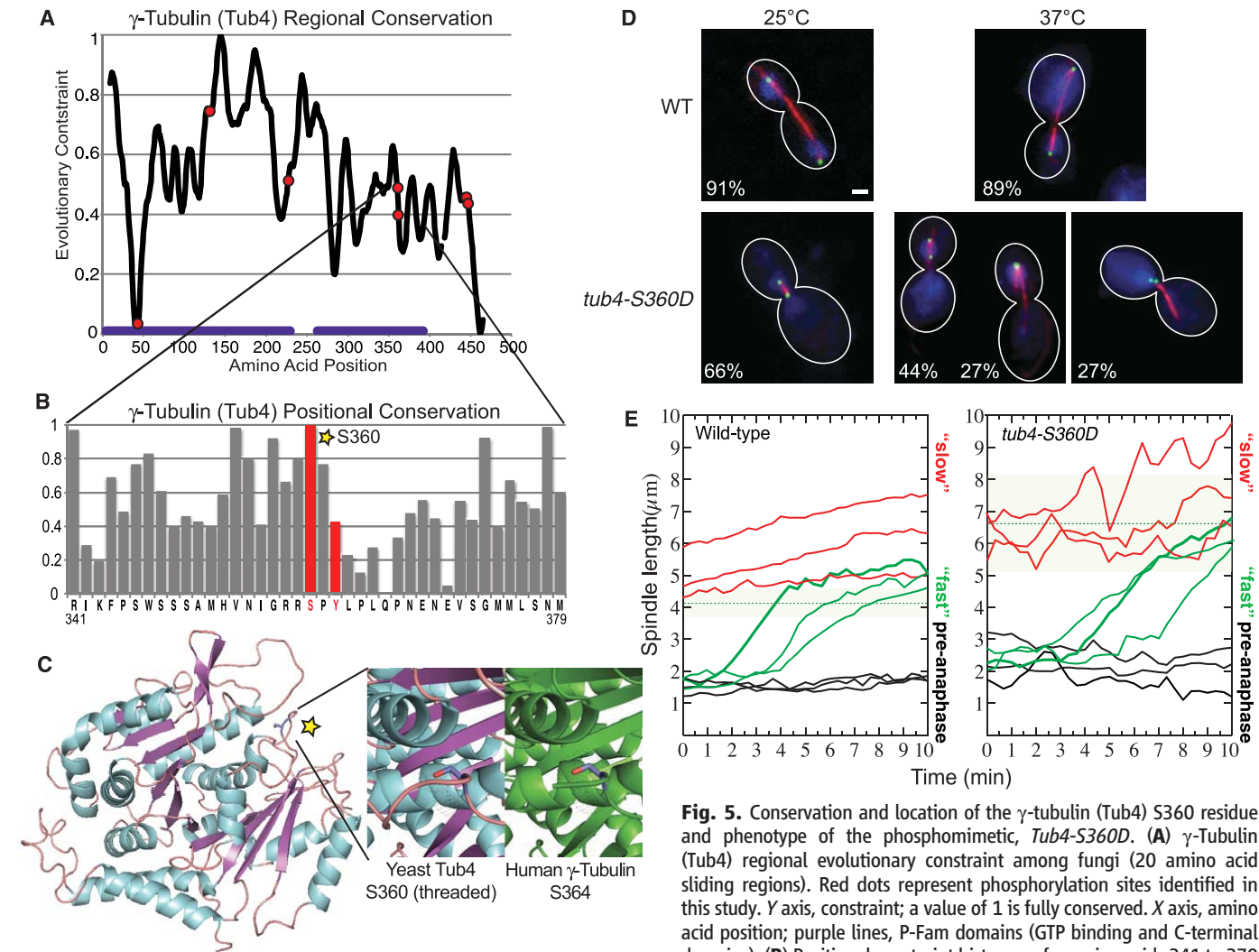


Fig. 5. Conservation and location of the γ -tubulin (Tub4) S360 residue and phenotype of the phosphomimetic, *Tub4-S360D*. (A) γ -Tubulin (Tub4) regional evolutionary constraint among fungi (20 amino acid sliding regions). Red dots represent phosphorylation sites identified in this study. Y axis, constraint; a value of 1 is fully conserved. X axis, amino acid position; purple lines, P-Fam domains (GTP binding and C-terminal domains). (B) Positional constraint histogram for amino acids 341 to 379. (C) Comparative model of yeast Tub4, generated by PyMOL (www.pymol.org), threaded onto the x-ray crystallographic structure of human γ -tubulin (4). Star marks position of Tub4-S360 in an exposed loop, expanded in inset and compared with human S364. (D) Immunofluorescence of mitotic spindles from WT and *tub4-S360D* cells at 25°C and 37°C. Centrosomes (Spc42-GFP, green), microtubules (anti- α -tubulin, red), and DNA [4',6'-diamidino-2-phenylindole (DAPI), blue] are shown. % indicates the percentage of cells with shown spindle structure, $n = 200$ cells for each. Scale bar, 1.5 μ m. (E) Live cell analysis of spindle length for WT and *tub4-S360D* cells at 25°C. Centrosomes labeled by Spc42-GFP (cyan fluorescent protein). Each time step is 20 s. Spindle length (μ m) measured by mother and daughter pole displacement (4). Spindle lengths are shown in black (metaphase), green (fast anaphase), and red (slow anaphase) and are representative for WT ($n = 17$) and *tub4-S360D* ($n = 18$) strains. Green hashed line is mean transition length, and green shaded shows standard deviation.

(*mad2Δ*) that allows for correction of mitotic defects, and by deletion of the EB1 homolog *BIM1* (*bim1Δ*), which is involved in microtubule dynamics (fig. S9A).

These genetic interactions suggested that *tub4-S360D* cells had defects in mitotic spindle assembly, which we analyzed by immunofluorescence microscopy and live cell imaging. At 25°C the majority (66%) of spindles in *tub4-S360D* large-budded mitotic cells had not extended past metaphase length [$\sim 1.5\ \mu\text{m}$ (24)], whereas WT cells (91%) had normal elongated anaphase spindles [6 to 10 μm (24)] (Fig. 5D, 25°C). This phenotype was exacerbated at 37°C, with 98% of *tub4-S360D* cells containing either adjacent or unresolvable spindle poles (54%) or metaphase-length spindles (44%) (Fig. 5D), compared with WT cells (89% normal anaphase spindles). Live cell analysis of microtubules in *tub4-S360D* cells grown at 25°C revealed that spindles persisted longer in the fast phase (25) of anaphase spindle elongation (Fig. 5E, green lines), resulting in a transition to the slow phase (25) with longer anaphase spindles [$6.6\ \mu\text{m} \pm 1.5$ (SEM)] than WT cells [$4.1\ \mu\text{m} \pm 0.4$ (SEM); $P < 0.001$] (Fig. 5E, dashed green lines). In addition, large spindle length fluctuations were observed in *tub4-S360D* cells before anaphase (Fig. 5E, black lines; quantified in fig. S10) and in the slow phase of anaphase (Fig. 5E, red lines). Thus, phosphorylation of a single Cdk site in γ -tubulin appears to contribute to proper dynamics of anaphase spindle microtubules.

Phosphoregulation of the centrosome is likely to be conserved, not only with respect to the protein kinases but also through specific residues in the respective human homologs. Illustrated by our analysis of γ -tubulin and Spc42, the conserved residues and phosphorylation patterns in yeast will be useful tools for studying the human centrosome, a much larger (>100 proteins) and more complicated microtubule organizing center.

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19. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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Supporting Online Material

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Mutagenic Processing of Ribonucleotides in DNA by Yeast Topoisomerase I

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The ribonuclease (RNase) H class of enzymes degrades the RNA component of RNA:DNA hybrids and is important in nucleic acid metabolism. RNase H2 is specialized to remove single ribonucleotides [ribonucleoside monophosphates (rNMPs)] from duplex DNA, and its absence in budding yeast has been associated with the accumulation of deletions within short tandem repeats. Here, we demonstrate that rNMP-associated deletion formation requires the activity of Top1, a topoisomerase that relaxes supercoils by reversibly nicking duplex DNA. The reported studies extend the role of Top1 to include the processing of rNMPs in genomic DNA into irreversible single-strand breaks, an activity that can have distinct mutagenic consequences and may be relevant to human disease.

The exclusion and removal of single ribonucleotides [ribonucleoside monophosphates (rNMPs)] from DNA are important for the stability and function of the genome. In *Saccharomyces cerevisiae*, the introduction of an rNMP-permissive form of DNA polymerase ϵ into a strain lacking ribonuclease (RNase) H2 confers a mutator phenotype and is associated with the ac-

cumulation of a distinct mutation class: deletions within short [2- to 5-base pairs (bp)] tandem repeats (1). In contrast to similar mutations initiated by DNA polymerase slippage during genome replication, however, the rNMP-associated deletion intermediates are not substrates for the postreplicative mismatch repair machinery (2). A similar deletion signature is associated with

high levels of transcription in yeast and requires the activity of Top1 (3, 4), a type 1B topoisomerase important for removing transcription-associated supercoils (5). Here, we demonstrate that rNMP-associated deletions are likewise dependent on Top1 activity, map in vitro the positions of Top1 cleavage at deletion hotspots identified in vivo, and confirm that Top1 has endoribonuclease activity when an rNMP is substituted at the scissile phosphate.

The *CAN1* gene encodes arginine permease, the loss of which confers resistance to the toxic arginine analog canavanine (Can-R phenotype). To determine the effect of persistent rNMPs on *CAN1* mutagenesis in yeast, we deleted the *RNH201* gene, which encodes the catalytic subunit of RNase H2 (6, 7). Though there was only a small elevation in the Can-R rate in the *rnh201* background, there was a substantial change in the corresponding mutation spectrum, with $\sim 40\%$ of mutations

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being deletions of 2- to 5-bp (partial and complete spectra are presented in Fig. 1 and fig. S1, respectively). To examine whether Top1 activity is relevant to rNMP-associated mutagenesis, we deleted the *TOP1* gene from the *rnh201* background. The rate of short deletions in the double mutant reverted to that observed in the wild-type strain, demonstrating that Top1 is required for the rNMP-associated deletion signature. Hereafter, we focus on the 2-bp deletion class, which localizes to discrete hotspots that coincide with short dinucleotide repeats. Some of these hotspots appear to be unique to the *rnh201* background, whereas others are Top1-dependent hotspots previously observed under high-transcription conditions [for instance, the (AG)₄ and (TC)₃ runs at nucleotides 254 and 1448 of *CAN1*, respectively] (3).

Small (20- to 30-bp) fragments containing representative hotspots identified in the *CAN1* reporter were transplanted into the *lys2ΔA746NR*

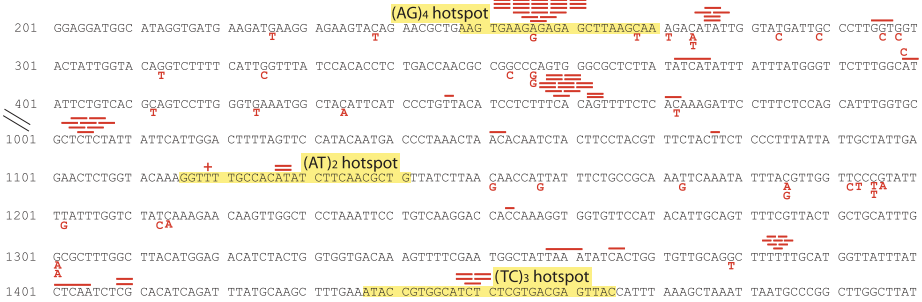
frameshift reversion assay, which detects net 1-bp insertions (8). The (AG)₄ and (TC)₃ hotspots were examined in this context, as well as the transcription-dependent (AT)₂ hotspot at position 1127 (3). A substantial proportion (21%) of *lys2ΔA746NR*:(AG)₄ revertants contained a 2-bp deletion at the introduced hotspot (gray bars in Fig. 2; see table S1 and fig. S2 for corresponding rates and spectra, respectively). Recapitulating what was seen in the *CAN1* assay, deletion of *RNH201* had the greatest effect on the (AG)₄ hotspot, elevating the Lys⁺ rate ~60-fold and shifting the spectrum toward events at the introduced hotspot (from 21 to 95%). In the *rnh201* background, the reversion rate of the *lys2ΔA746NR*:(TC)₃ allele was elevated sevenfold, and the proportion of mutations at the hotspot increased from 51 to 89%. At both the (AG)₄ and (TC)₃ hotspots, the 2-bp deletions were absent in the *rnh201 top1* double mutant, confirming their dependence on Top1. In

contrast to the (AG)₄ and (TC)₃ hotspots, loss of Rnh201 had little, if any, effect on activity of the Top1-dependent (AT)₂ hotspot. We suggest that either the rNMPs are not incorporated as often near the (AT)₂ hotspot or the rNMPs incorporated here are refractory to RNase H2 activity.

Loss of Rnh201 greatly elevates 4-bp deletions within a 4-bp tandem repeat in the *lys2ΔBgl* assay (9), an assay that queries the same sequence as the *lys2ΔA746NR* system, but detects net 1-bp deletions (10). Although it was suggested that the 4-bp deletions reflect defective Okazaki-fragment processing, their molecular similarity to 2-bp deletions in the *CAN1* assay prompted us to examine their dependence on Top1. Whereas the 4-bp deletions made up 69% of the spectrum in an *rnh201* mutant, they were completely eliminated upon additional deletion of *TOP1* (Fig. 2, fig. S2D, and table S1).

The Top1-dependent deletions identified under high-transcription conditions were proposed to reflect repair of trapped Top1 cleavage complexes (3, 4). In such complexes, the enzyme is linked to DNA via a 3'-phosphotyrosyl bond, leaving a 5'-OH on the other side of the nick (Fig. 3A). To explain the observed deletion pattern, it was

A *rnh201Δ* (N=185)



B *rnh201Δ top1Δ* (N=165)



C

Strain	Mutation rate (x 10 ⁻⁸)				
	BS	1-bp indel	2-5 bp del	Other	Total
WT (N=82)	8.3	0.9	0.2	0.6	10 (7.7-13)
<i>rnh201Δ</i> (N=185)	10.4	4.2	10.4	1.0	26 (22-32)
<i>rnh201Δ top1Δ</i> (N=165)	12.4	4.5	0.2	0.9	18 (17-22)

Fig. 1. Mutagenesis in the *CAN1* forward mutation assay. (A and B) Partial mutation spectra of Can-R mutants (complete spectra are shown in fig. S1). Nucleotides are numbered beginning with the ATG start codon. Base substitutions and indels are in red below and above the sequence, respectively; lengths of red bars correspond to deletion sizes. Sequences transplanted into the *lys2ΔA746NR* assay are highlighted in yellow. (C) Rates of individual mutation types at *CAN1*. N, number of mutants sequenced; indel, insertion/deletion; BS, base substitution; WT, wild type. 95% confidence intervals are in parentheses below total rates.

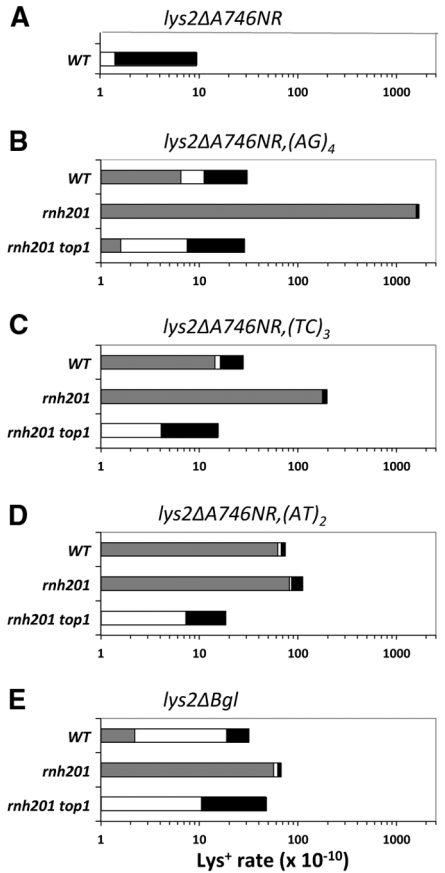


Fig. 2. (A to E) Rates of mutation types in *lys2* frameshift reversion assays. Gray bars correspond to 2- to 4-bp deletions at the introduced hotspot. White and black bars represent 1-bp indels and all other classes of mutations, respectively.

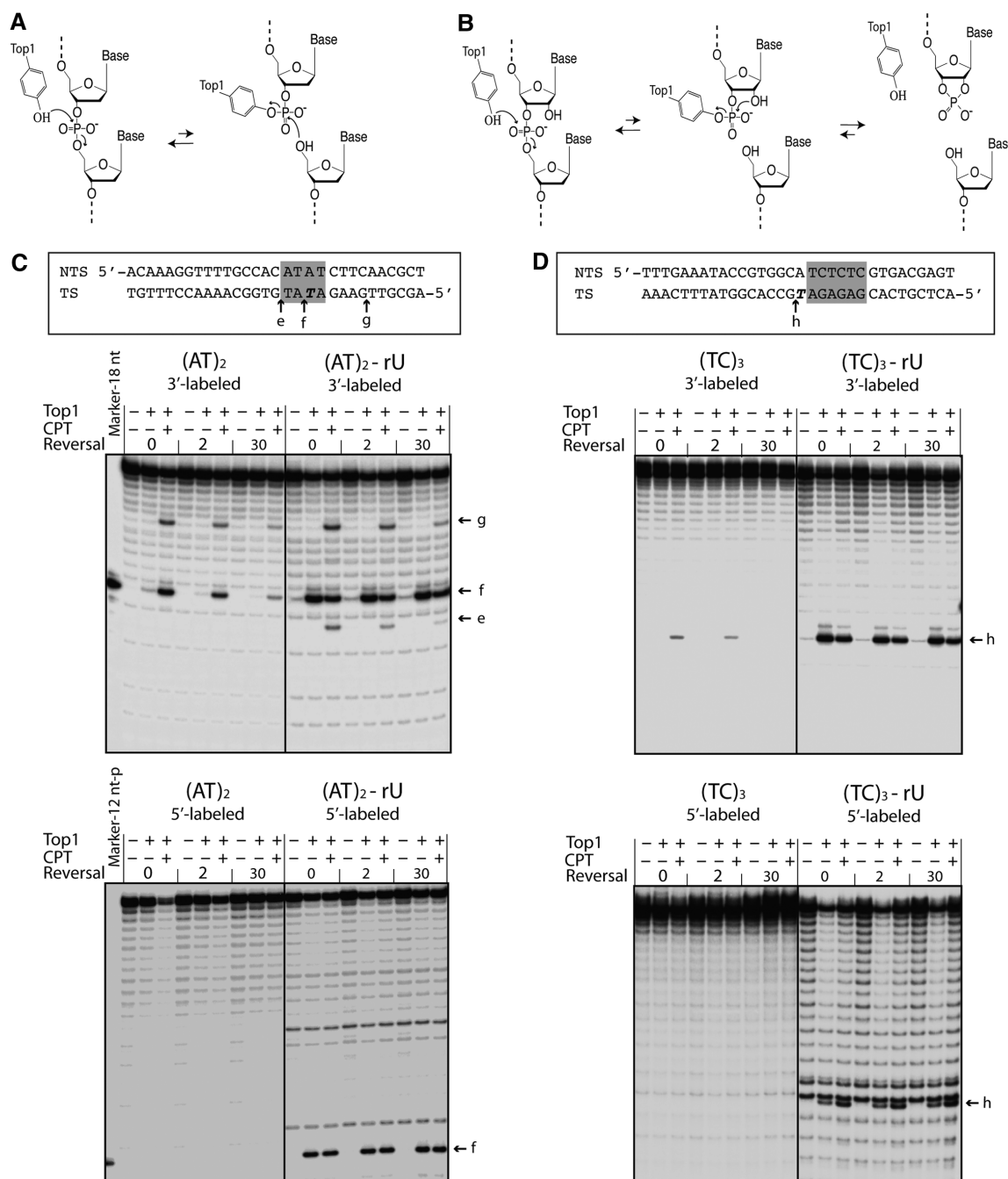
suggested that the Top1-generated ends are processed into a gap corresponding in size to that of the ensuing deletion (Fig. 4). The importance of the tandem repeat is that it can stabilize misalignment between complementary DNA strands, thereby bringing the ends flanking the gap together and facilitating their ligation. With regard to the Top1-dependent deletions observed here in the absence of RNase H2, biochemical studies have demonstrated that Top1 can act as an endonuclease if an rNMP is present at the cleavage site (11). In this reaction, the 2'-OH of the ribose attacks the 3'-phosphotyrosyl linkage between the enzyme and ribonucleotide, releasing Top1 and

leaving a 2',3'-cyclic phosphate end (Fig. 3B). In a manner analogous to that described above for a trapped cleavage complex, we suggest that subsequent processing of the cyclic end could generate a gap within the relevant tandem repeat, followed by misalignment between complementary strands to facilitate ligation (Fig. 4).

The molecular scenarios outlined above require Top1 cleavage either within or immediately adjacent to the tandem repeats where deletions occur. The small size and degeneracy of the Top1 consensus sequence, however, make it difficult to predict cleavage sites (12, 13), and mapping cleavage sites *in vitro* can be problematic

because of the rapid re-ligation of Top1-cleaved DNA. The Top1-specific drug camptothecin (CPT) is thus often used to stabilize the covalent cleavage intermediate, which can then be reversed in a time-dependent manner after an increase in salt concentration (14, 15). We examined CPT-stabilized Top1 cleavage intermediates produced when ~30-bp substrates containing the (AT)₂, (TC)₃, or (AG)₄ hotspot (Fig. 3 and fig. S3) were radioactively labeled on one of the 3' ends. Under these conditions, Top1 cleavage produces labeled fragments shorter than full-length DNA. For the (AT)₂ construct, the most prominent product was an 18-nucleotide (nt) fragment ("f")

Fig. 3. Top1 cleavage assays. **(A and B)** Mechanism of Top1 cleavage at a scissile deoxyribonucleoside monophosphate versus rNMP, respectively. **(C and D)** Cleavage of (AT)₂ and (TC)₃ hotspot fragments, respectively, by human Top1. In the fragment sequences, relevant dinucleotide repeats are highlighted in gray and ribosubstituted nucleotides are in bold italics. Transcribed and nontranscribed strands are designated TS and NTS, respectively; the TS strand was radioactively labeled at either the 3' or 5' end, as indicated. Labeled arrows indicate positions of Top1 cleavage. Time points for the salt reversal experiments are in minutes. The letters e to h indicate the positions of Top1 cleavage in the duplex substrates, and the corresponding cleavage products are indicated on the gels.



in Fig. 3C), indicating a major Top1 cleavage site on the transcribed strand in the middle of the dinucleotide repeat.

Substituting ribo-uracil (rU) for deoxyribo-thymine (dT) at the major cleavage site in the (AT)₂ fragment enhanced production of the corresponding Top1-generated fragment, presumably because the enzyme was released before the backbone could be re-ligated (11). Inclusion of CPT enhanced Top1 cleavage complex formation at other CPT-dependent sites (sites g and e in Fig. 3C), thus competing with and reducing cleavage at the rU-substituted site (site f). Though the addition of high salt reduced the intensity of the CPT-dependent fragments, the fragment corresponding to cleavage at rU did not change, indicating irreversible nicking at this specific site. To further examine the nature of the Top1 cleavage products, the corresponding transcribed strand was labeled at its 5' end (Fig. 3C, bottom panel). With the nonribosubstituted fragment, the cleavage intermediate failed to enter the gel, consistent with covalent linkage of Top1 to the 3' end at the cleavage site. With the rU substrate, however, a 5'-labeled, 12-nt cleavage product with a terminal phosphate was generated, indicating release of Top1 after cleavage, as predicted ("f" in bottom panel of Fig. 3C).

Results of similar analyses with an ~30-bp duplex fragment containing the (TC)₃ hotspot are presented in Fig. 3D and fig. S3. With the (TC)₃

hotspot, a single CPT-stabilized, salt-reversible Top1 cleavage product was detected when the transcribed strand was labeled at the 3' end (fragment "h"); no cleavage product was seen when the 5' end was labeled. Substitution of rU for the relevant dT residue yielded an irreversible Top1 cleavage product of appropriate size when either the 3' or 5' end of the transcribed strand was labeled. The sole Top1 cleavage site detected in the (TC)₃ fragment is immediately adjacent to, but is not within, the dinucleotide repeat. This has potential relevance to the requisite end-processing step(s), as well as to the mechanism of deletion formation in vivo. Finally, we examined possible positions of Top1 cleavage in a fragment containing the (AG)₄ hotspot. In this case, two cleavage sites were detected on each strand (fig. S3). We speculate that the site immediately adjacent to the (AG)₄ repeat on the transcribed strand is the one most likely relevant to Top1-dependent mutagenesis.

Here we have shown that, in addition to its well-known role in relaxing DNA supercoils, Top1 can initiate the removal of rNMPs from yeast genomic DNA. Because the intermediates formed during mutagenesis associated with rNMP incorporation by DNA polymerase ϵ are not substrates for mismatch repair (2), they probably arise outside the context of normal DNA replication and potentially could be a source of mutagenesis in terminally differentiated cells. Elucidating the pathway(s)

that resolves the cyclic end produced when Top1 incises at an rNMP is clearly of importance, as resolution/repair can be highly mutagenic in yeast and is expected to be similarly mutagenic in other organisms. There are a large number of proteins/complexes that have been implicated in the processing of CPT-stabilized Top1 intermediates that become trapped during DNA replication (16), some of which may be relevant to the processing that we predict occurs at Top1-generated nicks. Alterations in RNase H2 are one cause of the severe autoimmune disease Acardi Goutières syndrome (17), which is characterized by neurological dysfunction similar to that associated with congenital viral infection. An intriguing possibility is that the RNase H2-like activity of Top1 might have relevance to disease etiology. Finally, the mutagenic activity of Top1 identified here could be relevant to cases of mismatch repair-independent microsatellite instability described in human tumor cells (18).

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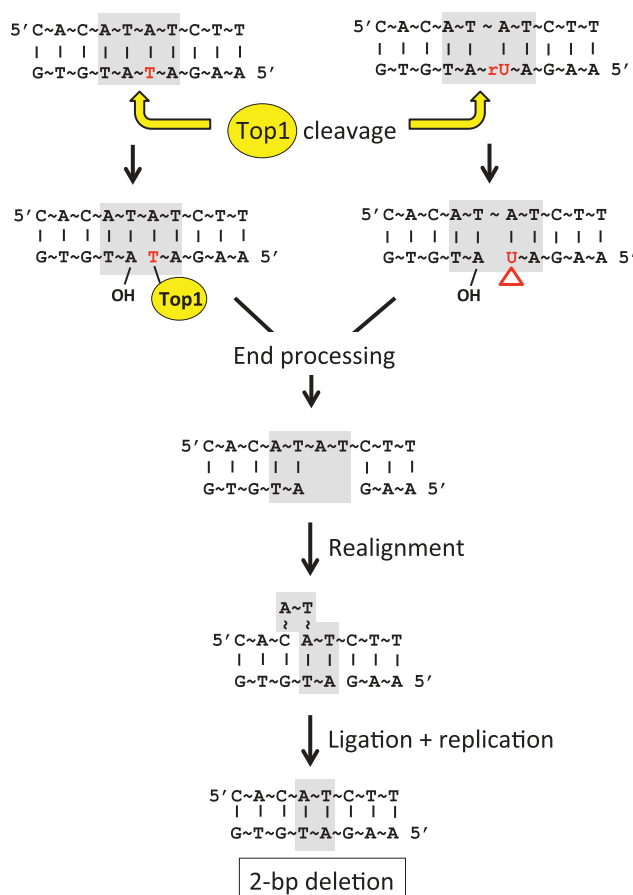
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Figs. S1 to S3
Table S1
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Fig. 4. Model for Top1-initiated deletions. The (AT)₂ hotspot sequence is shown: The top strand is the nontranscribed strand and the dinucleotide repeat highlighted in gray. The cyclic 2',3'-phosphate formed by Top1 cleavage at rUMP is indicated by a red triangle.



A Synthetic Optogenetic Transcription Device Enhances Blood-Glucose Homeostasis in Mice

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Synthetic biology has advanced the design of genetic devices that can be used to reprogram metabolic activities in mammalian cells. By functionally linking the signal transduction of melanopsin to the control circuit of the nuclear factor of activated T cells, we have designed a synthetic signaling cascade enabling light-inducible transgene expression in different cell lines grown in culture or bioreactors or implanted into mice. In animals harboring intraperitoneal hollow-fiber or subcutaneous implants containing light-inducible transgenic cells, the serum levels of the human glycoprotein secreted alkaline phosphatase could be remote-controlled with fiber optics or transdermally regulated through direct illumination. Light-controlled expression of the glucagon-like peptide 1 was able to attenuate glycemic excursions in type II diabetic mice. Synthetic light-pulse–transcription converters may have applications in therapeutics and protein expression technology.

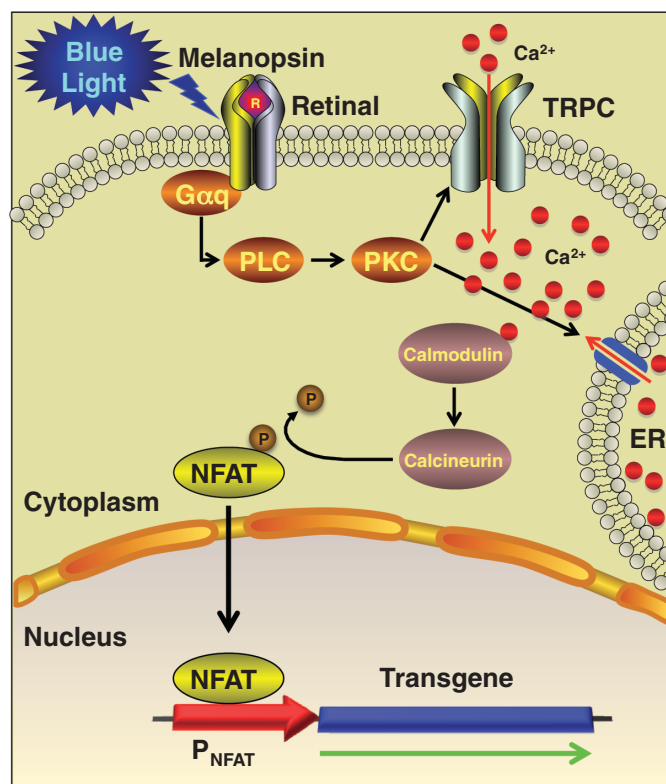
Light provides the energy to drive all metabolic processes and enables most multicellular life forms to see and adapt to their environment (1, 2). The mammalian retina is a light-capturing multicellular assembly (3) that controls the environment-brain interface, facilitating vision (4–6) and a variety of non-image-forming activities, such as the entrainment of circadian rhythms (7, 8). Image-forming and non-image-forming activities exhibit differential processing dynamics (1, 9). Whereas pattern recognition processed by the rods and cones is optimized for rapid (microsecond range) and sensitive spatial and temporal contrast acuity (10), photoentrainment requires light stimuli of high irradiance (over 200 times brighter than cones) and long duration (over 30 s) in order to align the biological clock with the dawn/dusk cycle and remain unaffected by local fluctuations in light exposure as the animal moves through its environment (3, 11).

Photoentrainment is processed by intrinsically photosensitive retinal ganglion cells (ipRGCs) expressing the photopigment melanopsin, a member of the opsin subgroup of G protein-coupled receptors that is typically linked to a chromophore consisting of a specific form of vitamin A called 11-cis-retinal (or related compounds) (12, 13). Although the exact mechanism of chromophore isoform processing, the specific types of G proteins involved, and the channels activated remain largely elusive, melanopsin was shown to trigger a phosphodiesterase-dependent cascade resulting in a calcium response in ipRGC somata that correlates with the action potential when illuminated by blue light (1, 3, 14). With its relatively high-brightness and long-exposure requirement,

melanopsin seems to be an ideal light-input component for the design of a synthetic mammalian light-controlled transcription device (15). By rewiring the melanopsin-induced intracellular calcium increase to calcium-dependent activation of calcineurin and calcineurin-mediated mobilization of the transcription factor nuclear factor of activated T cells (NFAT), which initiates transcription from specific promoters (16), illumination of mammalian cells could be directly linked

to transgene expression (Fig. 1). Simple cotransfection of several rodent and human cell lines with the constitutive melanopsin expression vector pHY42 (P_{hCMV}-melanopsin-pA_{SV40}) and the P_{NFAT}-driven luciferase reporter construct pGL4.30 (P_{NFAT}-luc2P-pA_{SV40}) showed that luciferase was exclusively induced whenever the cells were exposed for 24 hours to blue-light pulses (5 s ON and 10 s OFF, light power of 1.5×10^{18} photons s⁻¹ m⁻²) (Fig. 2A) (17). Differences in the availability of the promiscuous melanopsin-compatible G proteins and the efficiency of the intrinsic NFAT signaling pathway may in part explain the differences in basal expression and induction profiles among different cell lines. Despite capitalizing on the endogenous NFAT signaling pathway, basal transcription remains rather low in the absence of light, which indicates that pleiotropic input into this endogenous signaling cascade is insubstantial under standard culture conditions. Human embryonic kidney (HEK) 293 cells showed the best light-triggered transgene expression profile and were therefore used in all follow-up studies. A basic set of control experiments showed that (i) light was indeed increasing intracellular calcium levels (fig. S1, A and B); (ii) light-triggered transgene expression can be dose-dependently repressed by the addition of calcium-channel blockers, such as lanthanum chloride and ethylene glycol tetraacetic acid (fig. S1, C and D); (iii) the illumination regime had no negative impact on cell viability,

Fig. 1. Synthetic photo-transduction cascade. Blue-light (~480 nm)-mediated photo-isomerization of the 11-cis retinal (R) chromophore changes the conformation of melanopsin, which sequentially activates the Gαq-type G protein (Gαq), phospholipase C (PLC), and phosphokinase C (PKC). This triggers the influx of Ca²⁺ by activation of transient receptor potential channels (TRPCs) and possibly also from intracellular storage organelles, such as the endoplasmic reticulum (ER). The blue-light-triggered intracellular Ca²⁺ surge is linked to the signaling pathway of NFAT via the calcium sensor protein calmodulin. Calmodulin activates the serine/threonine phosphatase calcineurin, which dephosphorylates the serine-rich region and serine-proline repeats in the amino terminus of NFAT. This activates a conformational change that exposes a nuclear localization signal, results in nuclear import, and enables binding of NFAT to specific promoters (P_{NFAT}) and co-operation with resident transcription factors to induce transgene expression.



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which is considered critical because exposure of cell culture medium to light could lead to the production of toxic radicals and destruction of vital additives (fig. S1E) (18); and (iv) supplementation of the culture medium with the chromophore retinal is essential for melanopsin-mediated light responsiveness (fig. S1F) (12, 13, 17).

When HEK-293 cells were cotransfected with pHY42 (P_{hCMV} -melanopsin- pA_{SV40}) and pHY30 (P_{NFAT} -SEAP- pA_{SV40}) encoding P_{NFAT} -driven expression of the human placental secreted alkaline phosphatase (SEAP), and exposed for 48 hours to 5-s ON/10-s OFF blue-light pulses, the SEAP

levels had already reached near-maximum levels after 24 hours (Fig. 2B and fig. S2A). The maximum SEAP levels were comparable with those achieved by triggering the NFAT response, artificially using the ionophore ionomycin (Fig. 2B). This suggests that the light-induced melanopsin-triggered cascade was taking optimal advantage of the endogenous components of the NFAT pathway and may have been therefore operating at its maximum efficiency. Similar light-triggered expression kinetics could be visualized by means of fluorescence microscopy when using pHY41 (P_{NFAT} -EYFP- pA_{SV40}) instead of pHY30

(Fig. 2C). The SEAP levels of pHY30-/pHY42-cotransfected HEK-293 could be precisely fine-tuned by varying the irradiation times between 0 and 72 hours, which confirms that the synthetic control device is able to integrate the intensity of a pulsing light source over time and produce a sustained irradiance-adjustable transcription output (fig. S3). Exposure of engineered cells to a light-pulse of defined intensity and time period could also be used to precisely preprogram SEAP expression kinetics and predetermine the maximum SEAP levels reached after several days (Fig. 2D). Because light exposures of 3 to 24 hours lead to similar maximum SEAP levels, a standard 3-hour illumination is sufficient for optimal product gene expression (Fig. 2D). The 3-hour illumination provides sustained SEAP expression that rapidly declines after 24 hours and can be reversibly kicked on by 3-hour blue-light pulses at 24-hour intervals (Fig. 2E).

The production of protein pharmaceuticals that impair the growth or viability of their production cell lines, such as cancer therapeutics, represents a major biopharmaceutical manufacturing challenge (19, 20). Expression of these difficult-to-produce protein therapeutics requires precise titration of production levels into the viability and growth range of the production cell lines (20). Although many small-molecule-responsive transgene control systems are available to adjust product protein expression, the presence of trigger molecules in the production environment creates downstream-processing challenges and regulatory concerns (19, 21). We therefore evaluated light-triggered product gene expression in a standard bioreactor setting (17). The illumination hardware used to irradiate the transgenic production cell lines was identical to that used for the basic cell culture experiments. Over a 10-day bioreactor run, the production kinetics of the human glycoprotein SEAP could be programmed in a noninvasive manner simply by using a blue-light source placed outside the bioreactor (Fig. 3 and fig. S2B). Thus, product gene expression can be externally con-

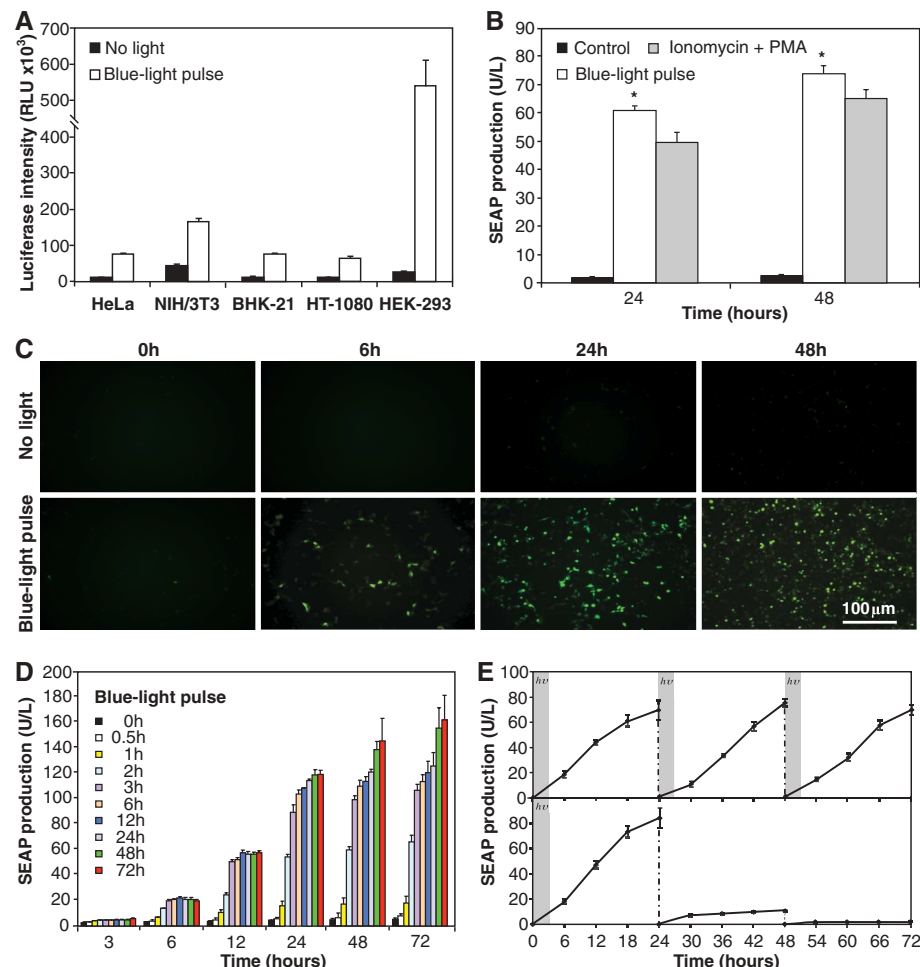


Fig. 2. Light-inducible transgene expression in mammalian cells. (A) Blue light-inducible intracellular luciferase expression levels in different mammalian cell lines 24 hours after cotransfection with pHY42 (P_{hCMV} -melanopsin- pA_{SV40}) and pGL4.30 (P_{NFAT} -luc2P- pA_{SV40}) and illumination for 24 hours with standard blue-light pulses. Data are mean $\pm$ SD; $n = 4$ independent experiments. (B) SEAP expression levels in the culture supernatant of HEK-293 cotransfected with pHY42 and pHY30 (P_{NFAT} -SEAP- pA_{SV40}) and either continuously illuminated with blue-light pulses or treated for the same time with 1 μmol ionomycin plus 10 ng/ml phorbol-myristate-acetate (PMA) to short-circuit Ca^{2+} signaling. Control cells were kept in the dark. Data are mean $\pm$ SD; $n = 4$ independent experiments. * $P < 0.05$. (C) Fluorescence micrographs profiling EYFP expression of HEK-293 cotransfected with pHY42 and pHY41 (P_{NFAT} -EYFP- pA_{SV40}) and illuminated with blue-light pulses for up to 48 hours. Control cells were kept in the dark. (D) SEAP expression profiles of pHY30-/pHY42-cotransfected HEK-293 illuminated with blue-light pulses for different periods of time. Data are mean $\pm$ SD; $n = 4$ independent experiments. (E) Reproducibility and reversibility of light-triggered transgene expression. pHY30-/pHY42-cotransfected HEK-293 were illuminated with blue-light pulses for 3 hours (hv, gray bar), and SEAP production was profiled for 72 hours. Every 24 hours, the culture medium was exchanged, the cell density was adjusted to 1×10^6 , and the cells were illuminated with and without additional 3-hour blue-light pulses. Data are mean $\pm$ SD; $n = 4$ independent experiments.

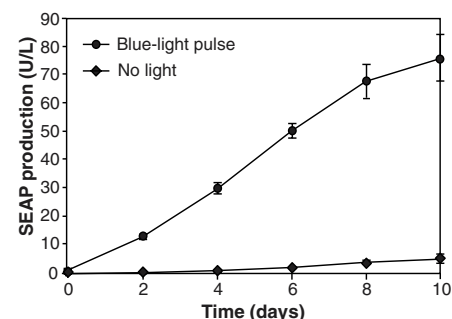


Fig. 3. Light-inducible product gene expression in bioreactors. 5×10^7 HEK-293 (200 ml, 2.5×10^5 cells/ml) transgenic for pHY42 (P_{hCMV} -melanopsin- pA_{SV40}) and pHY30 (P_{NFAT} -SEAP- pA_{SV40}) were seeded into roller bottles, and SEAP production was profiled for 10 days in the presence and absence of blue-light pulses. Data are mean $\pm$ SD; $n = 4$ independent experiments.

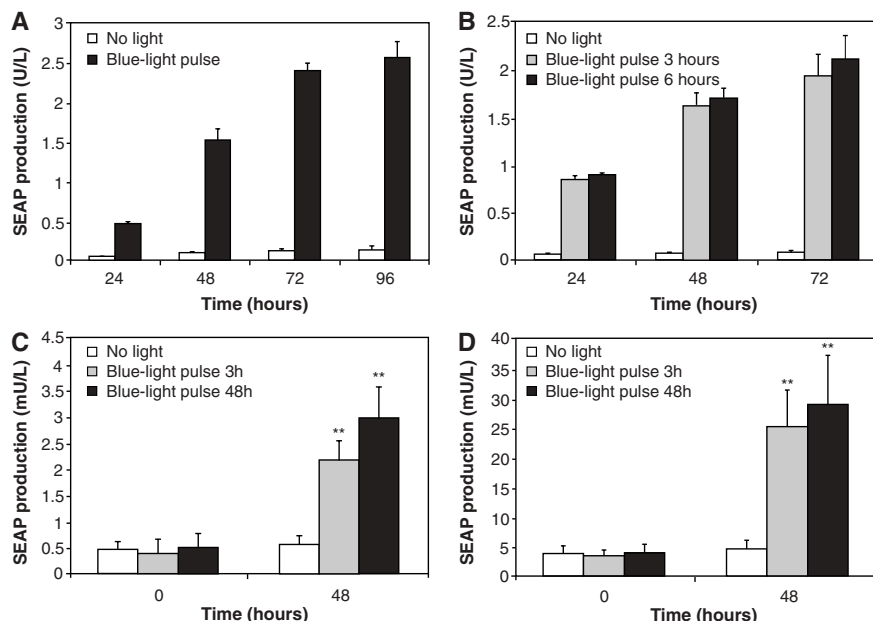


Fig. 4. Light-inducible transgene expression in mice. **(A)** SEAP production of hollow-fiber implants containing 1×10^5 pHY30-pHY42-transgenic HEK-293 maintained for up to 96 hours in 2 ml of culture medium and continuously illuminated via the optical fiber with blue-light pulses. Nonilluminated implants were used as the control. Data are mean $\pm$ SD; $n = 4$ independent experiments. **(B)** SEAP production of hollow-fiber implants containing 1×10^5 pHY30-pHY42-transgenic HEK-293 maintained for up to 72 hours in 2 ml of culture medium and illuminated via the optical fiber with blue-light pulses for 0, 3, or 6 hours. Data are mean $\pm$ SD; $n = 4$ independent experiments. **(C)** SEAP levels in the serum of mice containing optical-fiber-connected implants that were illuminated with blue-light pulses for 3 or 48 hours. Mice harbouring nonilluminated implants were used as the control. Data are mean $\pm$ SEM; statistics by two-tailed t test; $n = 8$ mice. **(D)** SEAP levels in the serum of mice containing subcutaneous implants of 1×10^6 microencapsulated pHY30-pHY42-transgenic cells that were illuminated with blue-light pulses for 3 or 48 hours. Data are mean $\pm$ SEM; statistics by two-tailed t test; $n = 8$ mice. ****** $P < 0.005$.

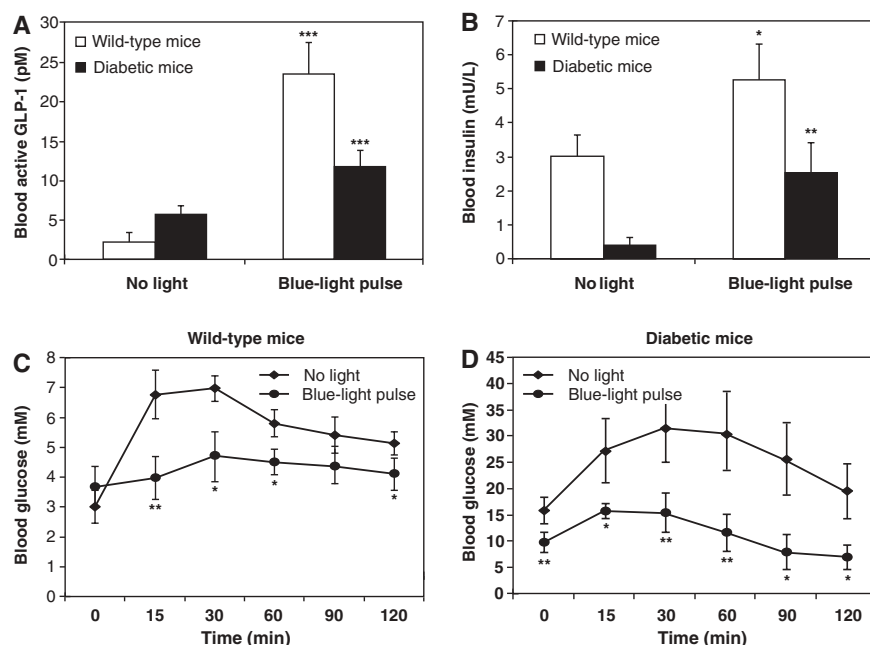


Fig. 5. Light-inducible expression of shGLP-1 in wild-type and diabetic db/db mice. **(A to C)** Wild-type and **(A), (B), and (D)** diabetic db/db mice subcutaneously implanted with 1×10^7 microencapsulated pHY42-pHY57-transgenic HEK-293 cells were illuminated with blue-light pulses for 48 hours (control mice received no light) and then profiled for **(A)** blood active GLP-1 and **(B)** insulin levels as well as **(C)** and **(D)** for glucose tolerance. Data are mean $\pm$ SEM; statistics by two-tailed t test; $n = 8$ mice. ***** $P < 0.05$, ****** $P < 0.005$, ******* $P < 0.0001$.

trolled, and production is triggered in cells as they move through a light beam. Also, the optogenetic transcription device shows improved product gene expression because it allows tight conditional expression of the highly toxic Rip death domain (fig. S4) (22, 23).

A molecule-free electromagnetic trigger device to control transgene expression in vivo is particularly appealing for future therapeutic applications. Because fiber optics could deliver light of the desired wavelength and intensity to an intracorporeal site, we used optical fibers (200 μ m in diameter) for lossless delivery of blue-light pulses to connected custom-designed intraperitoneal hollow-fiber implants containing 1×10^5 pHY30-pHY42-transgenic HEK-293 engineered for light-triggered SEAP expression (fig. S2C) (17). Preliminary in vitro studies showed that the optical fiber could optimally trigger SEAP expression in connected hollow-fiber implants maintained in culture medium for several days when using a continuous light-pulse regime with a standard blue-light intensity validated in previous cell culture experiments (Fig. 4A). Illuminating the cells contained in hollow fiber implants via connected optical fibers for a limited period of 3 or 6 hours resulted in almost identical SEAP production profiles for 3 days, indicating that a 3-hour irradiation period is sufficient to program the cells to produce the desired heterologous protein for several days (Fig. 4B). To validate light-triggered product gene expression in vivo, HEK-293 variants engineered for light-controlled SEAP expression contained in sealed hollow-fiber packs and connected to optical fibers were intraperitoneally implanted into mice and subsequently irradiated for 3 or 48 hours. The implants in control mice were not exposed to any light. The resulting SEAP levels quantified after 48 hours in the serum of both treatment groups confirmed the light-triggered remote control of product gene expression in animals (Fig. 4C). We also tested whether external blue-light pulses could transdermally control transgene expression in subcutaneous implants (17). When mice subcutaneously implanted with pHY30-pHY42-transgenic HEK-293 cells were illuminated for 3 or 48 hours with blue-light pulses, the plasma SEAP levels could be controlled accordingly (Fig. 4D and fig. S2D).

To validate light-triggered transcription control in a prototype therapeutic setting, we engineered HEK-293 cells for constitutive melanopsin production (pHY42) and P_{NEFAT}-driven expression of a glucagon-like peptide-1 variant (shGLP-1; pHY57) (17) that has displayed potent glucose homeostasis-modulating characteristics and may have potential in treating type II diabetes (24, 25). After control experiments confirmed the light-triggered expression of shGLP-1 and validated its capacity to induce insulin secretion in β -TC-6 cells (fig. S5), we implanted 1×10^7 microencapsulated pHY42-pHY57-transgenic HEK-293 cells subcutaneously into wild-type and diabetic db/db mice and illuminated the treated animals for 48 hours

with standard blue-light pulses (the control group was not exposed to blue-light pulses). shGLP-1 (Fig. 5A) and the resulting insulin (Fig. 5B) levels were significantly increased in wild-type as well as in diabetic db/db mice after blue-light exposure, and the action of both proteins significantly reduced the glycemic excursion of treated animals after intraperitoneal administration of glucose (wild-type mice, Fig. 5C; diabetic db/db mice, Fig. 5D). On the basis of these glucose tolerance tests showing the improvement of glucose homeostasis, and recent reports suggesting that the glucose-dependence of shGLP-1 automatically shuts down insulinotropic actions upon reaching normal glucose levels and so prevents hypoglycemia (25), light-triggered expression of shGLP-1 may be considered for the treatment and prevention of glucose-related pathologies (25, 26).

From plants to mammals, light-based energy and information is captured via receptors and processed via ion-based membrane potential (2, 3, 27, 28). Some of these native light receptors, such as melanopsin (3) and channelrhodopsin (29), have been extensively used for heterologous intervention in native neuron-triggered activities in order to understand the photoentrainment of the circadian clock (7) or to restore visual function in retinal degeneration (5, 6, 29, 30). Capitalizing on the principles of synthetic biology to assemble functional biologic devices from well-characterized components in a rational and predictable manner (31), it has recently become possible to engineer synthetic signaling cascades and control networks to program metabolic behavior (32–35), cell morphology (36), and therapeutic interventions (37) with high precision. By combining heterologous factors (melanopsin) and control modules (P_{NFAT}) with promiscuous complementary endogenous machineries (G proteins, NFAT pathway), we re-

wired melanopsin-mediated G protein-coupled receptor signaling to NFAT control, taking advantage of their common intracellular calcium-based, second-messenger-based signaling system as the interface (16). When engineered into mammalian cells grown in bioreactors or implanted into mice, this synthetic light-control device enabled conversion of a physiologically inert pulsed blue-light beam into a continuous transcription response, the level of which could be adjusted by the irradiation period. Remote control of transgene expression by means of electromagnetic waves may enable quantitative cell culture experiments, providing opportunities for economical manufacturing of difficult-to-express protein therapeutics and for low-risk dosing in gene- and cell-based therapies.

References and Notes

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Selective Attention from Voluntary Control of Neurons in Prefrontal Cortex

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Animals can learn to voluntarily control neuronal activity within various brain areas through operant conditioning, but the relevance of that control to cognitive functions is unknown. We found that rhesus monkeys can control the activity of neurons within the frontal eye field (FEF), an oculomotor area of the prefrontal cortex. However, operantly driven FEF activity was primarily associated with selective visual attention, and not oculomotor preparation. Attentional effects were untrained and were observed both behaviorally and neurophysiologically. Furthermore, selective attention correlated with voluntary, but not spontaneous, fluctuations in FEF activity. Our results reveal a specific association of voluntarily driven neuronal activity with “top-down” attention and suggest a basis for the use of neurofeedback training to treat disorders of attention.

Animal and human subjects can learn to alter their own brain activity when they are provided with feedback (1–4). Voluntary control of neuronal activity is likely associated with changes in behavior or cognitive functions, but that relationship is unclear. Oper-

ant control of motor cortical neurons is typically dissociated from movement production (5–8), and there are no clear behavioral consequences of operant control of neuronal spiking activity in other brain structures (1, 4). Naturally, one might ask whether a chosen control strategy can elicit

untrained behavioral or neurophysiological outcomes. To address this question, we examined the consequences of voluntary control of neurons in the frontal eye field (FEF), a visuomotor area within the prefrontal cortex with a known role in the programming of saccadic eye movements (9) and visual spatial attention (10), in rhesus monkeys (Fig. 1A).

We first asked whether the activity of FEF neurons can be controlled voluntarily by the monkey without explicit training on any task. We used an operant control paradigm (2) in which the monkey received juice rewards for alternately increasing and decreasing the firing rates (FRs) of FEF neurons during fixation (11) (Fig. 1, B and C). During trials, the monkey received auditory

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feedback via pure tones, the pitch of which corresponded to the instantaneous FR of multiunit activity (MUA) at a FEF recording site. In

blocks of “Up” trials, the monkey received a reward each time the FR, and thus the tone pitch, reached a high threshold (Fig. 1C). In blocks of

“Down” trials, the monkey was rewarded each time a low threshold was reached.

We recorded from 94 FEF sites in two monkeys (monkey B, 49; monkey C, 45) during operant control. Figure 2, A to C, shows the results of a representative experiment. At this site, the MUA FR on Up trials was greater than on Down trials (Fig. 2A; $P < 10^{-4}$), indicating that the monkey modulated the MUA in the rewarded direction. This FR difference persisted through the entire trial and was maintained between blocks of Up and Down trials (Fig. 2B). We quantified neuronal control with a “control index” (CI), with positive CIs indicating changes in activity in the rewarded direction. The overall CI for the example site was 0.064, corresponding to a 13.7% increase in FR from Down to Up trials (Fig. 2C).

Across all experiments, monkeys exerted modest but reliable control over the FRs of FEF neurons. The mean CI across sites was 0.031 (Fig. 2D; monkey B, $P = 0.0086$; monkey C, $P = 0.0010$; combined, $P = 10^{-4}$), corresponding to a 6.4% difference in FR. However, the magnitude of control varied considerably throughout the course of each experiment ($P = 0.0045$; fig. S1). Furthermore, for individual FEF sites we found both significant positive and negative effects of control (11). Fifty-five experiments (59%) showed individually significant effects of control; of these, 38 (69%) had positive CIs, with a mean of 0.080 ($P < 10^{-4}$), and 17 (31%) had

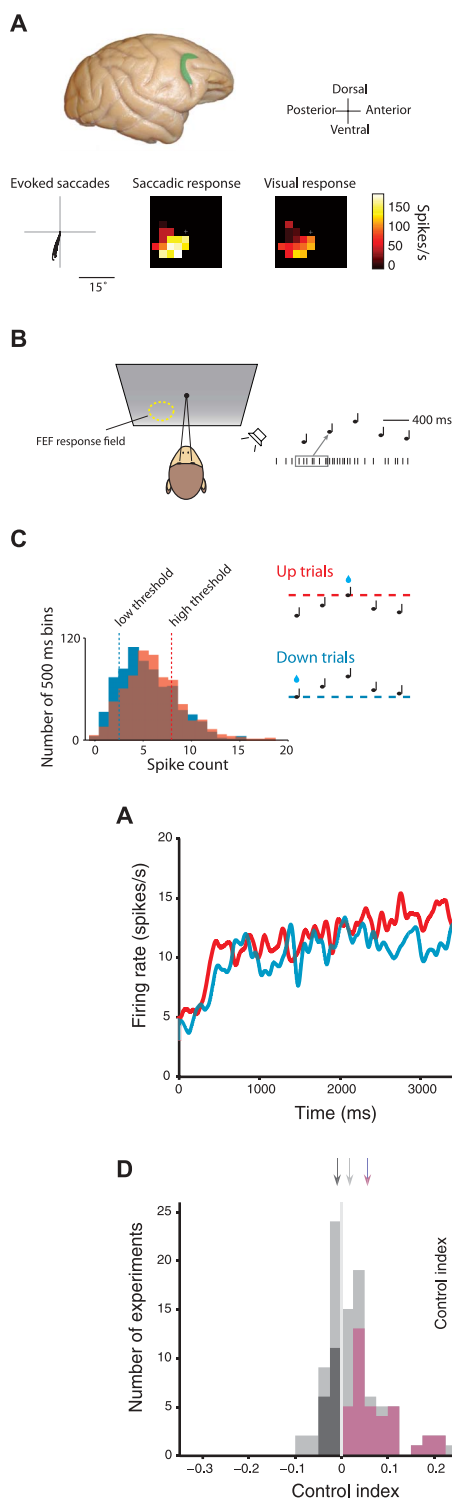


Fig. 2. Voluntary control of FEF neurons. (A) Average MUA over the course of Up (red) and Down (blue) trials during an example experiment. (B) Firing rates for each Down and Up trial in (A) are indicated by blue and red triangles, respectively, with the mean firing rate for a block of trials represented by a horizontal line. Gray vertical bars mark the first 10 trials after each block transition, which were excluded from analysis. (C) Time course of the control index for the experiment in (A). (D) Population histogram of multiunit CIs. Light

gray histogram shows all experiments; purple histogram shows experiments with individually significant positive control; dark gray histogram shows experiments with significant negative control. Inset shows the mean time course of voluntary control (colors as in the histogram). Thickness of each envelope is $\pm$ SEM. (E) The control index of single FEF neurons as a function of their visual (left) and saccadic (right) response indices. (F) Population histograms showing Up-Down differences in LFP power in four frequency bands. * $P < 0.05$, *** $P < 0.001$.

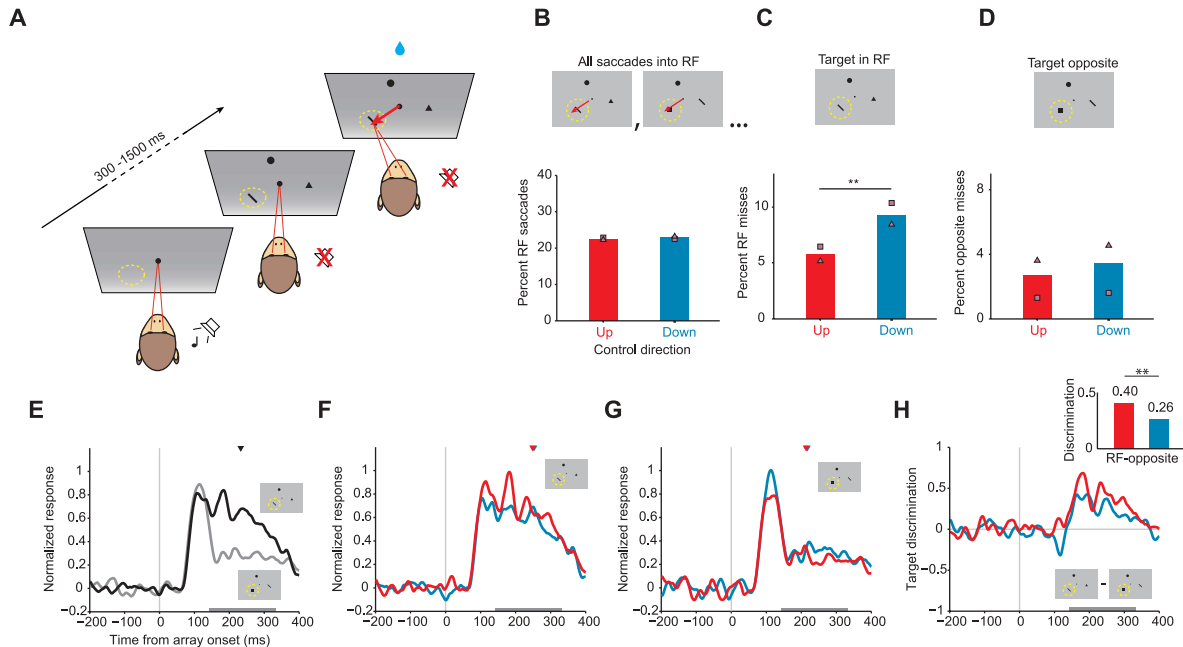
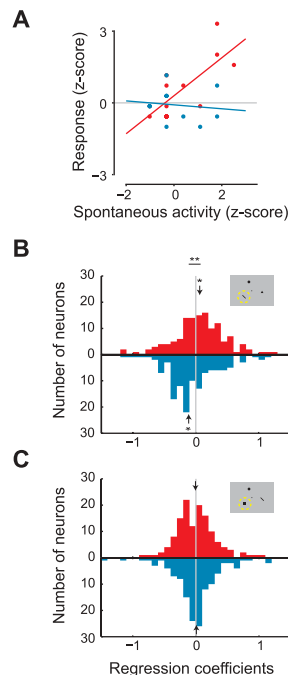


Fig. 3. Behavioral and physiological consequences of operant FEF control. (A) Visual search probe trials, in which a search array appeared and the monkey was rewarded (blue droplet) for directing a saccade toward an oriented bar target. (B) Percentage of probe trials in which a saccade was directed into the RF, correctly or incorrectly, during all conditions (ellipses) and during Up (red) and Down (blue) operant control. Purple triangles, monkey B; purple squares, monkey C. (C) Proportion of target misses in the RF. (D) Proportion of target misses opposite the RF. (E) FEF responses to the visual search array. The normalized population response of 150 FEF neurons aligned to array onset for trials with the target in

the RF (black) or opposite the RF (gray). Gray bar along abscissa indicates the interval during which the target and distractor responses were significantly different. (F) FEF responses to targets in the RF on Up and Down trials. Data are the same as the black line in (E), but red and blue lines show responses on Up and Down trials, respectively. (G) FEF responses to distractors in the RF, when the target was opposite. Data are the same as the gray line in (E). (H) Target discrimination by FEF neurons, defined as the difference in responses between “target in RF” and “target opposite” trials. Bar plot shows mean target discrimination on Up (red) and Down (blue) trials. $^{**}P < 0.01$.

Fig. 4. Correlation of spontaneous activity with FEF responses. (A) Linear regression between the probe trial response of an example FEF neuron and its spontaneous pre-probe activity during Up (red) and Down (blue) control. (B and C) Population histogram of regression coefficients describing the relationship between spontaneous activity and responses to targets (B) or distractors (C) in the RF. Arrows denote medians of each distribution. $^{*}P < 0.05$, $^{**}P < 0.01$.



negative CIs, with a mean of -0.018 ($P = 0.0002$). The effects observed in MUA were also present in isolated single neurons (11). We found no correlation between the CI and the visual ($P = 0.4385$) or motor ($P = 0.1204$) properties of the neurons, indicating that both visual- and movement-related

neurons (12, 13) could be operantly controlled (Fig. 2E). The FR effect was also accompanied by a difference in the power spectra of local field potentials (LFPs) at the recording site. LFP power in the beta (13 to 30 Hz) and gamma (30 to 70 Hz) bands increased during Up versus Down trials (β

median difference = 0.059 dB, $P = 0.027$; $\gamma = 0.048$ dB, $P = 0.00068$), whereas there was no difference for low-frequency theta (4 to 8 Hz) or alpha (8 to 13 Hz) bands (Fig. 2F; $\theta = 0.033$ dB, $P = 0.36$; $\alpha = 0.063$ dB, $P = 0.33$).

We wondered whether voluntary modulation of FEF activity might have associated effects on behavior or on the function of the controlled neurons. Neuronal control could be achieved through nonspecific means such as changes in general arousal or vigilance, or perhaps through means that were motor in nature but did not require a saccade (figs. S4 to S6). Alternatively, voluntary control might be achieved by a cognitive or behavioral strategy specific to the presumed role of FEF neurons (10, 14). We therefore used visual search trials to probe psychophysical and neuronal performance during operant control. On 29% of operant control trials, the monkey was presented with a visual search array at an unpredictable time (Fig. 3A). Feedback then ceased, and the monkey was no longer rewarded for controlling neuronal activity. Instead, the monkey received a reward for directing a saccade to the search target (an oriented bar) if it was present at any location, or for maintaining fixation if no target was present.

In 82 experiments (monkey B, 41; monkey C, 41), the mean probe trial performance was 86.3% correct (monkey B, 81.9%; monkey C, 90.7%). The overall percentage of saccades targeting the

response field (RF) was the same during Up and Down trials (Fig. 3B; Up = 22.6% of 4441, Down = 23.0% of 4346, $P = 0.65$; monkey B, $P = 0.43$; monkey C, $P = 0.80$). Likewise, the latencies of saccades to RF targets were similar during Up and Down trials (fig. S7; z -score normalized Up latency = -0.001 ± 0.033 ; Down = 0.001 ± 0.032 ; $P = 0.967$). Thus, we found no evidence that operant control was associated with saccade preparation. The saccadic main sequence was also unaltered by voluntary control (fig. S8).

In contrast, neuronal control had a spatially specific effect on visual search performance. The proportion of trials on which the monkey failed to detect the target in the RF ("misses") was significantly greater during Down trials in both monkeys (Fig. 3C; Up = 5.7% of 1098, Down = 9.3% of 1124, $P = 0.0017$; monkey B, $P = 0.020$; monkey C, $P = 0.032$). In contrast, the proportion of misses at locations opposite the RF was statistically indistinguishable between Up and Down trials (Fig. 3D; Up = 2.7% of 1120, Down = 3.4% of 1114, $P = 0.31$; monkey B, $P = 0.39$; monkey C, $P = 0.70$). The influence of voluntary control on search performance was confined to locations less than $\sim 6^\circ$ from the controlled neuron's RF (fig. S9). We confirmed that search performance was specifically correlated with the direction of voluntary control and not with spontaneous fluctuations in pre-probe neural activity (fig. S10) (17).

Next, we measured the effect of voluntary control on the ability of FEF neurons to identify the search target. As in previous studies (15), we found that the responses of FEF neurons ($n = 150$; monkey B, 61; monkey C, 89) could indicate whether a search target or a distractor appeared within the RF (Fig. 3E) (17). However, we also found that the response to RF targets was 16.5% greater on Up than on Down trials (Fig. 3F; Up mean peak-normalized rate = 0.668, Down = 0.573, $P = 0.0054$). In contrast, there was no difference between responses to distractors during Up versus Down trials (Fig. 3G; Up = 0.265, Down = 0.313, $P = 0.9207$). Thus, neuronal control selectively enhanced FEF responses to targets but not to distractors, resulting in a significant enhancement in target discrimination during Up trials relative to Down trials (Fig. 3H; Up = 0.404, Down = 0.259, $P = 0.004$; monkey B, $P = 0.046$; monkey C, $P = 0.037$).

As with the behavior, target discrimination by FEF neurons was specifically related to operant control rather than noncontrolled fluctuations in spontaneous pre-probe activity. Overall pre-probe activity for combined Up and Down trials did not predict the FEF responses to targets (median regression coefficient = 0.0145, $P = 0.9337$). However, dividing trials according to the direction of voluntary control revealed that pre-probe activity was positively correlated with the target response during Up trials (Fig. 4, A and B; median regression coefficient = 0.0633, $P = 0.0303$), but anticorrelated during Down trials (median = -0.1128 , $P = 0.0468$; Up versus Down, $P = 0.0033$). In contrast, responses to distractors were uncor-

related with pre-probe activity for both Up (Fig. 4C; median = -0.0072 , $P = 0.6560$) and Down trials (median = 1.05×10^{-17} , $P = 0.9853$; Up versus Down, $P = 0.7431$). Thus, pre-probe FR combined constructively with target-driven activity only during upward control.

Our results demonstrate that voluntary control of FEF neuronal activity is associated with spatially selective visual attention, measured behaviorally and neurophysiologically. In controlling FEF activity, monkeys converged on a strategy that dissociated spatial attention from saccade preparation. This untrained dissociation provides a naturalistic demonstration that the two functions are not wholly interdependent—a point that has proven difficult to substantiate (10). We also observed that the attentional consequences of operant control were correlated with voluntarily driven, rather than spontaneous, FEF activity. This selective linkage might occur if control alters interactions between the FEF and visual cortex—a possibility supported by the enhanced LFP power at frequencies with suspected involvement in long-range interactions during attention (16). Finally, our results suggest a basis for recent evidence that neurofeedback training may be therapeutic in patients with attention deficit-hyperactivity disorder (17, 18), as they demonstrate that voluntary modulation of neural activity can indeed produce specific changes in cognitive performance.

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Inducing Sleep by Remote Control Facilitates Memory Consolidation in *Drosophila*

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Sleep is believed to play an important role in memory consolidation. We induced sleep on demand by expressing the temperature-gated nonspecific cation channel *Transient receptor potential cation channel (UAS-TrpA1)* in neurons, including those with projections to the dorsal fan-shaped body (FB). When the temperature was raised to 31°C, flies entered a quiescent state that meets the criteria for identifying sleep. When sleep was induced for 4 hours after a massed-training protocol for courtship conditioning that is not capable of inducing long-term memory (LTM) by itself, flies develop an LTM. Activating the dorsal FB in the absence of sleep did not result in the formation of LTM after massed training.

Although the functions of sleep remain unknown, sleep is believed to be important for maintaining optimal performance in a large and diverse number of biological pro-

cesses (1, 2). Historically, the importance of sleep has been most convincingly established by demonstrating negative consequences that accrue in its absence (3). In contrast, methods that allow an experimenter to induce sleep on demand are lacking. Thus, it has been difficult to demonstrate that sleep serves a beneficial role per se. Studies in humans indicate sleep may play an active role in the strengthening or stabilizing of new memories (4, 5). With this in mind, we conducted experiments

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in *Drosophila* to determine whether it is possible to induce sleep on demand and thereby influence memory (6).

We expressed the bacterial sodium channel *NaChBac* (7, 8) under the control of several *GAL4* (*galactosidase 4*) drivers to constitutively activate specific neuronal circuits. Flies that displayed periods of quiescence lasting ≥ 5 min were further evaluated to determine whether they met the traditional criterion for identifying sleep (9, 10). Quiescence was dramatically increased when *UAS-NaChBac* (*NaChBac*; UAS indicates upstream activating sequence) was expressed un-

der the control of three independent *GAL4* drivers: *C5-GAL4*, *104y-GAL4*, and *C205-GAL4* (Fig. 1, A to C, and fig. S1) (11). The expression pattern for each of these *GAL4* drivers overlaps in neurons that strongly resemble *ExF12* cells, which are known to project to the dorsal fan-shaped body (FB) (Fig. 1C and fig. S2) (11). In contrast, the expression pattern of these three drivers did not show prominent overlap in neurons in other brain regions (fig. S2) (12). Thus, although we cannot formally exclude a role for brain regions outside the dorsal FB, it is likely that the dorsal FB plays a role in regulating quiescence.

Because expressing *UAS-NaChBac* with *C5-GAL4* and *104y-GAL4* resulted in the largest changes in both quiescence and in the consolidation of quiescent episodes, we focused on these two drivers (Fig. 1, D to F, and fig. S1B). Both *C5/+>NaChBac/+* and *104y/+>NaChBac/+* flies behaved normally and spontaneously aroused to engage in goal-directed behaviors. Moreover, the number of quiescent bouts did not differ between experimental lines and both of their parental controls, suggesting that the quiescent bouts are regulated (fig. S3). To determine whether the activation of the FB inhibited locomotion,

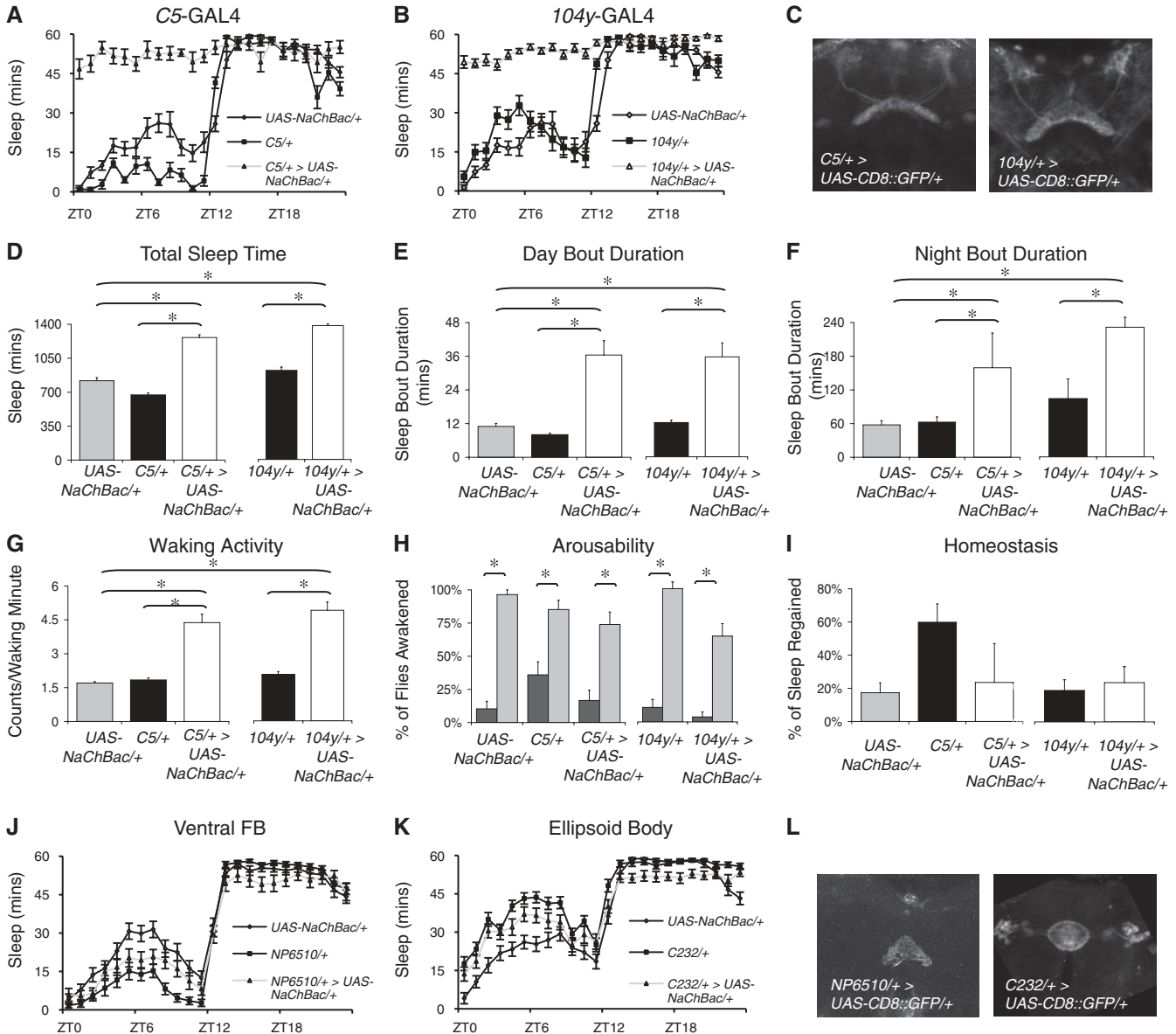


Fig. 1. Chronic sleep induction. (**A** and **B**) Expressing *UAS-NaChBac* using *C5-GAL4* or *104y-GAL4* increased sleep. Data presented as sleep in min/hour ($n = 12$ to 16 per group). Error bars indicate SEM; ZT, zeitgeber time. (**C**) *C5/+>UAS-CD8::GFP* and *104y/+>UAS-CD8::GFP* expression. (**D**) Total sleep was increased in *C5/+>UAS-NaChBac/+* and *104y/+>UAS-NaChBac/+* flies; $*P < 0.05$, modified Bonferroni test. (**E** and **F**) Neuronal activation with *C5* and *104y* increased daytime and nighttime sleep bout duration. (**G**) Intensity of waking activity was increased in *C5/+>UAS-NaChBac/+* and

104y/+>UAS-NaChBac/+ flies. (**H**) A light pulse during the night (gray) awakened all genotypes compared with undisturbed siblings. χ^2 , $n = 23$ to 29 each group, $*P < 0.05$; Mann-Whitney U test comparing each group to untreated siblings. (**I**) All genotypes exhibited a sleep rebound after 12 hours of sleep deprivation. (**J** and **K**) Expressing *UAS-NaChBac* using *NP6510-GAL4* or *C232-GAL4* did not change sleep. (**L**) *NP6510/+>UAS-CD8::GFP/+* labeled the ventral FB, whereas *C232/+>UAS-CD8::GFP/+* was expressed in the EB.

we examined the intensity of waking activity. *C5/+>NaChBac/+* and *104y/+>NaChBac/+* flies exhibited an increase in waking activity compared

with controls (Fig. 1G). To determine whether quiescent episodes were rapidly reversible, we exposed flies to a light pulse during the night;

all genotypes exhibited a significant increase in the percentage of arousals (Fig. 1H). Similarly, a shorter light pulse was less effective in inducing

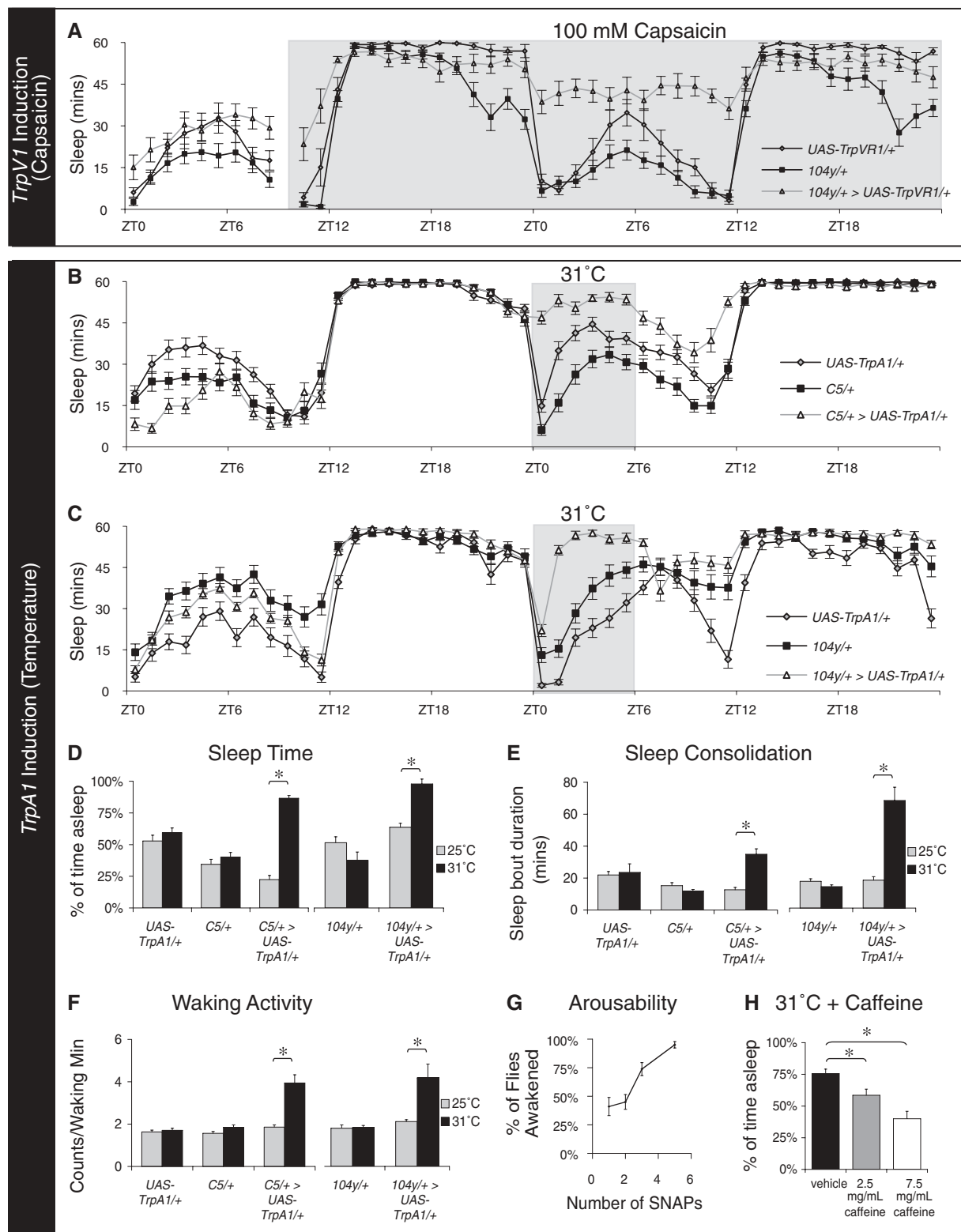


Fig. 2. Inducing sleep on demand. **(A)** Capsaicin-fed *104y/+>UAS-TrpVR1/+* flies increased sleep. **(B to D)** Transferring *C5/+>UAS-TrpA1/+* and *104y/+>UAS-TrpA1/+* flies to 31°C for 6 hours increased sleep. **P* < 0.05 modified Bonferroni test. **(E)** Mean sleep bout duration was increased in *C5/+>UAS-TrpA1/+* and *104y/+>UAS-TrpA1/+* flies at 31°C. **(F)**

Counts per waking minute increased at 31°C in *C5/+>UAS-TrpA1/+* and *104y/+>UAS-TrpA1/+* flies. **(G)** Awakenings increased with perturbation number. SNAP, sleep-nullifying apparatus. **(H)** *104y/+>UAS-TrpA1/+* flies fed caffeine (2.5 mg/ml or 7.5 mg/ml) 1 hour before 31°C exposure display weakened sleep induction.

wakefulness, indicating that quiescent episodes are associated with increased arousal thresholds (fig. S4). Moreover, all genotypes showed a rebound in quiescent episodes when kept awake for 12 hours, indicating the presence of homeostatic regulation. (Fig. 1I) (13). Lastly, we examined quiescence in response to caffeine and during the first days of adulthood to determine whether it would exhibit properties previously described for sleep. We found that caffeine disrupted quiescence in all genotypes (fig. S5). Moreover, the ontogenetic changes previously described for sleep were also observed for all genotypes (fig. S6). Thus, the observed quiescence episodes meet the historical criteria for sleep.

The central complex, comprising the FB and Ellipsoid Body (EB), is associated with locomotion and memory (12, 14, 15). To determine whether exciting neurons in the ventral FB or EB would also alter sleep, we expressed *NaChBac* in the ventral FB by using *NP6510-GAL4* and *NP6561-GAL4* and in the EB by using *C232-GAL4* and *78y-GAL4*. No sleep changes were observed (Fig. 1, J to L, and fig. S7). To test whether previously identified sleep-regulatory genes or known FB neuroanatomical pathways overlap with *104y*- and *C5-GAL4*-expressing neurons, we conducted immunohistochemical and genetic experiments (figs. S8 to S12) (14, 16–20). These experiments failed to identify candidate sleep-regulatory genes.

The ability to acutely control the timing and duration of sleep induction may be critical for elucidating the interaction between sleep and other processes such as memory formation. Therefore, we asked whether the *transient receptor potential* constructs *TrpVr1* and *TrpA1* could be used to induce sleep on demand (21, 22). Although *104y/+ > UAS-TrpVr1/+* flies showed an increase in sleep after being fed 100 mM capsaicin, the effect was not immediate (Fig. 2A). However, when the temperature-sensitive *TrpA1* allele (21) was expressed with *C5* and *104y-GAL4*, sleep and sleep consolidation were significantly increased when flies were moved to 31°C (Fig. 2, B to E). Although overall motor activity temporarily decreased during sleep induction (fig. S13), the intensity of waking activity was also increased, indicating that the acute activation did not impair locomotion (Fig. 2F). Moreover, exposing sleeping flies to increasing amounts of mechanical perturbation induced greater amounts of wakefulness, indicating the presence of increased arousal thresholds (Fig. 2G). To determine whether induced sleep could be antagonized by caffeine, *104y/+ > UAS-TrpA1/+* flies were fed caffeine for 1 hour before being placed at 31°C; caffeine attenuated the increase in sleep (Fig. 2H). Lastly, inducing sleep in *104y/+ > UAS-TrpA1/+* flies for 4 hours transcriptionally down-regulates the wake-associated genes *fatty acid synthase* (8) and *homer* (23), suggesting shared molecular characteristics with spontaneous sleep (fig. S14).

Recent studies have used sleep deprivation protocols to examine the role for sleep in synaptic

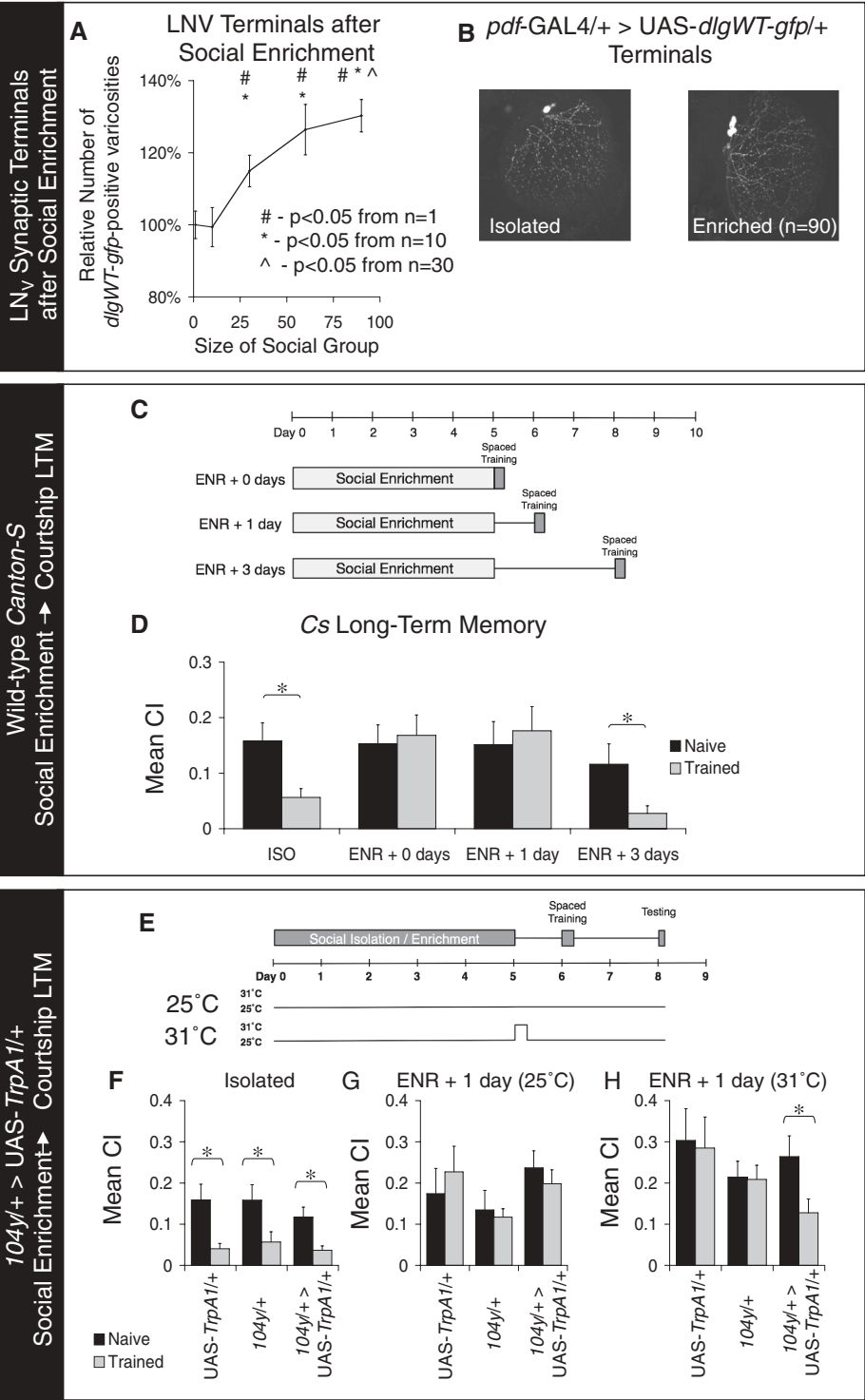


Fig. 3. Inducing sleep reverses deficits in LTM after social enrichment. **(A)** Quantification of *dlg*-positive terminals expressed as percent of isolated siblings ($n = 13$ to 16 brains per group). **(B)** Images of *dlgGFP*-positive terminals from LNVs in isolated flies and enriched siblings (~95 flies). **(C)** Schematic for evaluating LTM after social enrichment. **(D)** Isolated (ISO) flies reduced courtship 48 hours after training (Student's t test $P = 0.008$, $n = 40$ to 42). Enriched siblings lacked LTM (ENR+0 days, Student's t test $P = 0.77$, $n = 28$ or 29). Enriched flies that were isolated for 1 day before training showed no LTM (ENR+1 day, Student's t test $P = 0.61$, $n = 25$ to 29). Enriched flies that were isolated for 3 days exhibited LTM (ENR+3 days, Student's t test $P = 0.029$, $n = 12$ or 13). **(E)** Schematic for testing LTM recovery after sleep induction. **(F)** Isolated males displayed LTM. **(G)** ENR + 1 day (25°C) flies showed no LTM when housed at 25°C. **(H)** *104y/+ > UAS-TrpA1/+* flies that were enriched, housed at 31°C for 4 hours, then trained exhibited LTM; * $P < 0.05$ planned comparisons. Error bars indicate SEM.

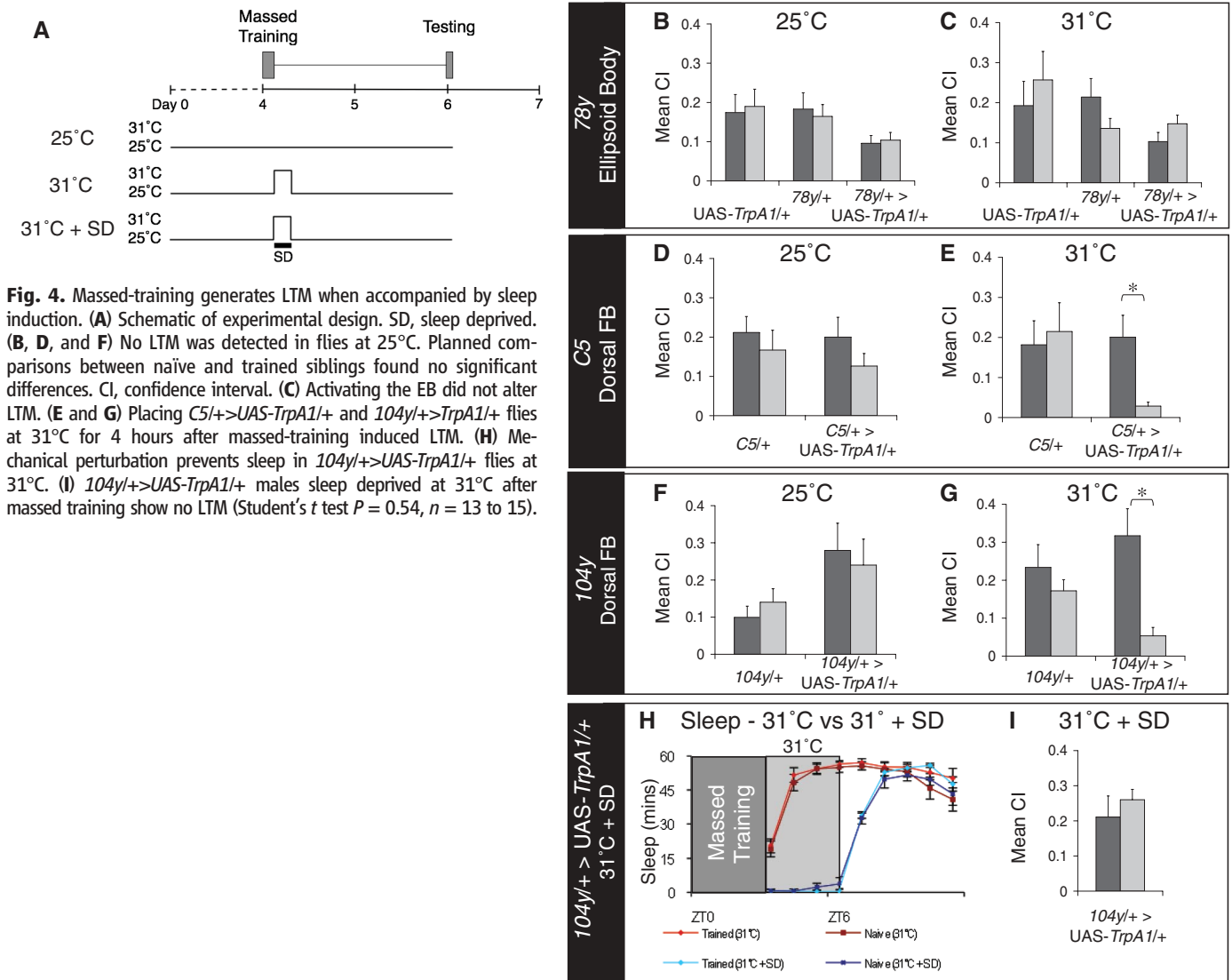


Fig. 4. Massed-training generates LTM when accompanied by sleep induction. **(A)** Schematic of experimental design. SD, sleep deprived. **(B, D, and F)** No LTM was detected in flies at 25°C. Planned comparisons between naïve and trained siblings found no significant differences. CI, confidence interval. **(C)** Activating the EB did not alter LTM. **(E and G)** Placing *C5/+>UAS-TrpA1/+* and *104y/+>UAS-TrpA1/+* flies at 31°C for 4 hours after massed-training induced LTM. **(H)** Mechanical perturbation prevents sleep in *104y/+>UAS-TrpA1/+* flies at 31°C. **(I)** *104y/+>UAS-TrpA1/+* males sleep deprived at 31°C after massed training show no LTM (Student's *t* test $P = 0.54$, $n = 13$ to 15).

downscaling and the consolidation of long-term memory (LTM) (20, 23–27). Can inducing sleep on demand provide additional insight into the role for sleep in these processes? First, we evaluated the synaptic homeostasis model, which hypothesizes that synaptic connections increase during waking and are downscaled during sleep; in the absence of sleep, neuronal circuits would exceed available space and/or saturate, thereby interfering with individual's ability to learn (28). Because housing flies in a complex social environment for 5 days increases the number of synaptic terminals in projections from the large ventral lateral neurons (LN_vs) (20), we asked whether social enrichment would impair LTM. We used *pigment-dispersing factor* (*pdf*)–*GAL4* to drive expression of a green fluorescent protein (GFP)–tagged construct of the postsynaptic protein *discs-large* (*UAS-dlgWT-gfp*) in flies exposed to increasingly larger social groups. The number of *dlg*-positive terminals increased with the size of the social group (Fig. 3, A and B). To determine whether the saturation of *dlg*-positive terminals

had functional consequences, male *Canton-s* (*Cs*) flies were socially enriched in a group of 90 flies and then exposed to spaced training for courtship conditioning (Fig. 3C). *Cs* flies that had been maintained in social isolation displayed normal LTM (Fig. 3D). However, enriched flies had no LTM when trained either immediately after enrichment or after 1 day of delay.

The impaired LTM was not attributable to overcrowding: Enrichment using only 45 flies also disrupted LTM, and flies enriched in constant darkness retained LTM (fig. S15). Consistent with our previous results showing that *dlg*-positive terminals return to baseline after 48 hours (20), LTM was observed in flies trained 3 days after enrichment (Fig. 3D, right).

According to the synaptic homeostasis model, sleep downscaling synapses and restores optimal learning. Thus, we hypothesized that inducing sleep immediately after social enrichment should restore LTM more quickly. Flies were housed in either social isolation or in a socially enriched group of ~95 flies. After 5 days, flies were placed

into individual tubes at either 25°C or 31°C for 4 hours. All flies were returned to 25°C and then trained for courtship conditioning the following morning; LTM was tested 2 days after training (Fig. 3E). All isolated flies exhibited LTM (Fig. 3F). No LTM was observed in any genotype when training was preceded by 5 days of enrichment and flies were maintained at 25°C (Fig. 3G). However, LTM was observed in *104y/+>UAS-TrpA1/+* flies when sleep was induced for 4 hours immediately after social enrichment (Fig. 3H). Reduced courtship cannot be attributed to nonspecific effects of FB activation or heat exposure because courtship is not altered in naïve *104y/+>UAS-TrpA1/+* flies or controls exposed to 31°C for 4 hours (Fig. 3G–H). Inducing sleep for 4 hours after enrichment not only accelerated LTM recovery but also reduced PDF-positive terminals in *104y/+>UAS-TrpA1/+* flies within 24 hours (fig. S16). Thus, our data are consistent with the synaptic homeostasis model and support a positive role for sleep in reversing LTM deficits induced by social enrichment.

To determine whether sleep plays a positive role in memory consolidation, we induced sleep for 4 hours immediately after a massed training protocol that does not result in the formation of LTM (Fig. 4A). Massed training did not induce LTM in any genotype at 25°C (Fig. 4, B, D, and F). Moreover, activating the EB did not alter LTM, indicating that neither heat exposure nor activating portions of the central complex that do not alter sleep facilitates memory consolidation (Fig. 4C). However, when sleep was induced after massed training by switching flies to 31°C for 4 hours, *C5/+>UAS-TripA1/+* and *104y-GAL4* flies displayed an LTM (Fig. 4, E and G). Thus, when flies were maintained at 25°C after massed training, no LTM was observed in any genotype. However, when sleep was induced for 4 hours, flies display LTM, even though the training protocol is not sufficient to induce memory consolidation by itself.

An alternative interpretation is that activating neuronal circuits enhances memory consolidation and that LTM would be observed even in the absence of sleep. Therefore, we sleep-deprived *104y/+>UAS-TripA1/+* flies while they were maintained at 31°C after training. Our sleep deprivation apparatus efficiently kept *104y/+>UAS-TripA1/+* flies awake, further emphasizing that neuronal activation does not simply result in a paralyzed or comatose-like state (Fig. 4H). Additionally, activation of neuronal activation in the absence of sleep does not result in the formation of LTM after massed training.

We identified a circuit that plays a role in sleep regulation and can be activated on demand to

precisely control the timing and duration of sleep. Our data complements previous sleep-deprivation experiments by demonstrating that sleep plays a positive role in both synaptic homeostasis and memory consolidation. Thus, inducing sleep facilitates the formation of LTM even for a training protocol that, by itself, is only able to induce short-term memory. We expect that the ability to acutely control the timing and duration of sleep induction will be critical for elucidating the interaction between sleep and other complex processes and ultimately the function of sleep.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/332/6037/1571/DC1

Materials and Methods

Figs. S1 to S16

Table S1

References (29–34)

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Sleep and Synaptic Homeostasis: Structural Evidence in *Drosophila*

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The functions of sleep remain elusive, but a strong link exists between sleep need and neuronal plasticity. We tested the hypothesis that plastic processes during wake lead to a net increase in synaptic strength and sleep is necessary for synaptic renormalization. We found that, in three *Drosophila* neuronal circuits, synapse size or number increases after a few hours of wake and decreases only if flies are allowed to sleep. A richer wake experience resulted in both larger synaptic growth and greater sleep need. Finally, we demonstrate that the gene *Fmr1* (*fragile X mental retardation 1*) plays an important role in sleep-dependent synaptic renormalization.

Sleep is present in every species that has been carefully studied (1), including *Drosophila melanogaster* (2, 3), but its functions remain elusive. Increasing evidence points to a link between sleep need and neuronal plasticity (1, 4, 5). A recent hypothesis (6) suggests that a consequence of staying awake is a pro-

gressive increase in synaptic strength, as the awake brain learns and adapts to an ever-changing environment mostly through synaptic potentiation (7). However, such increase would soon become unsustainable, because stronger synapses consume more energy, occupy more space, require more supplies, and cannot be further potentiated, saturating the ability to learn. Thus, according to the synaptic homeostasis hypothesis, sleep may serve an essential function by promoting a homeostatic reduction in synaptic strength down to sustainable levels. Also, the hypothesis

predicts that the more one learns and adapts (i.e., the more intense is the wake experience), the more one needs to sleep. Findings in rodents are consistent with this hypothesis. For instance, molecular and electrophysiological markers of synaptic strength are higher after wake and lower after sleep (8, 9). Moreover, presynaptic terminals of hypocretin neurons in zebrafish larvae undergo both circadian and sleep-wake-dependent structural changes, the latter consistent with sleep-dependent down-regulation (10). Finally, in the fly brain, overall levels of synaptic proteins increase after wake and decrease after sleep (11), and synaptic structural changes have been described after very long sleep deprivation (12). These results suggest that a role for sleep in synaptic homeostasis may hold in phylogenetically distant species and may thus be of general importance.

The evidence in support of the synaptic homeostasis hypothesis is mainly correlative, and thus it is important to seek direct proof that sleep is necessary for synaptic renormalization and to do so at the level of individual synapses. Moreover, the synaptic homeostasis hypothesis predicts that behavioral paradigms that enhance wake-related plasticity in specific neural circuits should increase synaptic strength in those circuits as well as sleep need, but this prediction has never been tested.

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Finally, the cellular mechanisms that underlie synaptic and sleep changes remain unexplored. We exploited the power of *Drosophila* genetics, combined with confocal microscopy and behavioral analysis, to address these questions.

Changes in synaptic strength are often associated with changes in synaptic structure, including synapse number and size, although the link between structural and functional plasticity is complex (13–15). In mammals, the diameter and length of synaptic spines correlate with the size of the postsynaptic density and with the magnitude of electric signals transmitted to the dendritic shaft (16, 17). Moreover, the induction of synaptic potentiation leads to growth of synapses and spines, whereas synaptic depression causes synapses and spines to retract or shrink (13–15). Similarly, in *Drosophila*, synaptic morphology at

the neuromuscular junction changes depending on experience, and these changes correlate with synaptic strength (18). Previous *in vivo* experiments in mammals and flies measured overall changes in electrophysiological and molecular markers of synaptic strength, without cellular resolution, and without direct evidence for morphological changes in synaptic terminals. We selected three specific cell populations in the fly brain and asked whether sleep and wake affect synaptic density and size.

The first cell group we studied included the small ventral lateral neurons (LNvs), a subset of circadian oscillator neurons that are part of the wake promoting system (19) and express the neuropeptide pigment dispersing factor (PDF) (20) (Fig. 1A). To visualize changes in presynaptic morphology, we expressed a fusion protein be-

tween synaptotagmin and enhanced GFP (syt-eGFP), whose protein product colocalizes with native synaptic vesicles (21). We also measured PDF expression, because the latter is another marker of presynaptic boutons in small LNvs (22). First, we tested adult females (7 days old) collected either during the light period after 7 hours of mainly (>75%) spontaneous wake or during the dark period after 7 hours of mostly sleep (>80%) or sleep deprivation (>90%) (Fig. 1B). Syt-eGFP and PDF staining were both higher in the presynaptic region of sleep-deprived and spontaneously awake flies relative to sleeping flies (Fig. 1, C and D), whereas no differences were found in the axonal processes extending from the cell bodies to the presynaptic region ($P = 0.3$ for both syt-eGFP and PDF, Kruskal-Wallis test), suggesting that the changes are independent

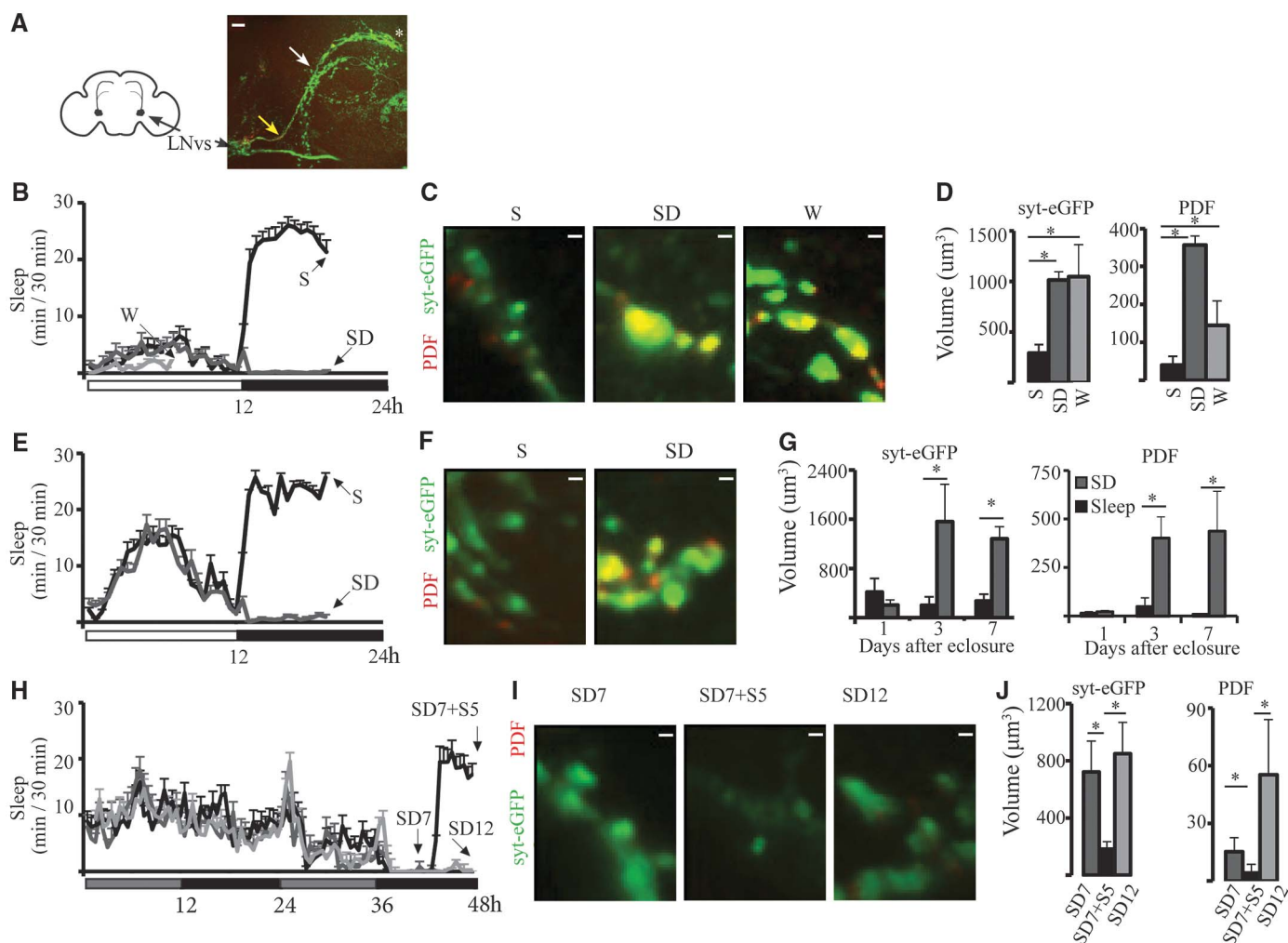


Fig. 1. Sleep/wake presynaptic changes in small LNvs. (A) (Left) Schematic frontal section of fly brain with LNvs neurons projecting to the dorsal brain. (Right) Example of small LNvs axonal terminals stained for syt-eGFP (green). Yellow and white arrows point to where LNvs axons leave the posterior optic tract and to the first axonal bifurcation, respectively. Asterisk marks the tip of the terminal region whose volume was measured, as shown in (C), (F), and (I). (B) Mean sleep duration in 7-day-old females used for imaging after spontaneous wake (W), sleep deprivation (SD), or sleep (S). Horizontal white and black bars indicate light and dark periods, respectively. (C) Examples of small LNvs axonal

terminals stained for syt-eGFP (green), PDF (red, overlap yellow), and volume measurements (D) in females (S = 9, W = 9, SD = 5). (E) Mean sleep duration in 7-day-old males used for imaging. (F) Examples of axonal terminals in males. (G) Mean volume measurements in males harvested 1, 3, and 7 days after eclosure (N = 5 per time point). (H) Mean sleep duration in *Per⁰¹* males kept in constant darkness. At the onset of the second subjective night, flies underwent SD for 7 or 12 hours, or 7 hours of SD followed by 5 hours of sleep. (I) Examples of axonal terminals. (J) Mean volume measurements (N = 7 per group). Scale bars, 10 μm in (A), 1 μm in (C), (F), and (I). All panels show mean ± SEM.

of circadian time and specific to the presynaptic terminal. We then tested males; because they have less consolidated wake during the day than females, we only collected flies at night, after sleep or sleep deprivation (Fig. 1E). Sleep-deprived 3- and 7-day-old males consistently showed higher presynaptic syt-eGFP and PDF staining than sleeping flies (Fig. 1, F and G). In contrast, 1-day-old flies showed low syt-eGFP and PDF staining after both sleep and sleep deprivation (Fig. 1, F and G). The lack of PDF staining in very young flies suggests that these neurons are still inactive soon after eclosion. Moreover, because PDF promotes arousal, low PDF staining is consistent with flies being predominantly asleep after eclosion (3), even if mechanical stimulation was used to try to keep them awake, consistent with high sleep need and elevated arousal threshold in newborn mammals. Syt-eGFP staining did not change in newly eclosed flies, whose PDF levels were very low. Syt-eGFP and PDF expression were also measured in *Per⁰¹* flies carrying a null mutation of the clock gene *Period*. Because *Per⁰¹* mutants have no spontaneous consolidated sleep, flies were collected immediately after 7 hours of sleep deprivation or after 5 additional hours of either recovery sleep or

sleep deprivation (Fig. 1H). Overall, syt-eGFP and PDF staining in presynaptic terminals was reduced in *Per⁰¹* mutants relative to wild-type (WT) flies but was still high after both 7 and 12 hours of sleep deprivation and low after recovery sleep (Fig. 1, I and J).

The second cell group we analyzed included γ neurons of the mushroom bodies (Fig. 2A, inset), because they can be targeted by mosaic analysis with a repressible cell marker (MARCM) to visualize single cells (23), show a relatively simple morphology, and undergo activity-dependent pruning (24). Moreover, the mushroom bodies are involved in sleep regulation (25, 26), and mutations altering cyclic adenosine monophosphate-dependent protein kinase signaling or *Fmr1* (*fragile X mental retardation 1*) expression in these brain regions affect both sleep need and experience-dependent structural plasticity (12, 27–29). Flies were collected at night after 7 hours of sleep or sleep deprivation (Fig. 2A), and dissected brains were immunostained for GFP-tagged CD8 to visualize neuronal membranes (Fig. 2B). We found that the axonal tips were larger after sleep deprivation than after sleep (Fig. 2, C and D), consistent with an increase in volume of presynaptic terminals. To confirm this result, we gen-

erated fly stocks with γ MARCM clones expressing syt-eGFP, and flies were collected after 7 hours of mostly spontaneous wake, or during the dark period after 7 hours of mostly sleep or sleep deprivation (Fig. 2E). As expected, syt-eGFP tended to accumulate in puncta along lightly stained processes (Fig. 2F), in contrast to the diffuse CD8-GFP staining (Fig. 2B). Syt-eGFP puncta were larger in sleep deprived and spontaneously awake flies relative to sleeping flies (Fig. 2, G and H).

Next, we studied whether postsynaptic morphological changes also occur as a function of sleep and wake. To do so, we focused on the first giant tangential neuron of the lobula plate vertical system (VS). This cell (VS1) (Fig. 3A) is unambiguously recognizable, and its stereotyped dendritic tree shows small actin-enriched protrusions morphologically and functionally similar to mammalian dendritic spines (30). We compared flies that were spontaneously awake during the day or that slept or were sleep deprived during the first 7 hours of the night (Fig. 3B). Single VS1 spines were visualized using an antibody against actin-GFP and counted in one easily identifiable branch (Fig. 3, A and C) (see Supporting Online Material). The total number of spines was similar in spontaneously awake and sleeping flies but

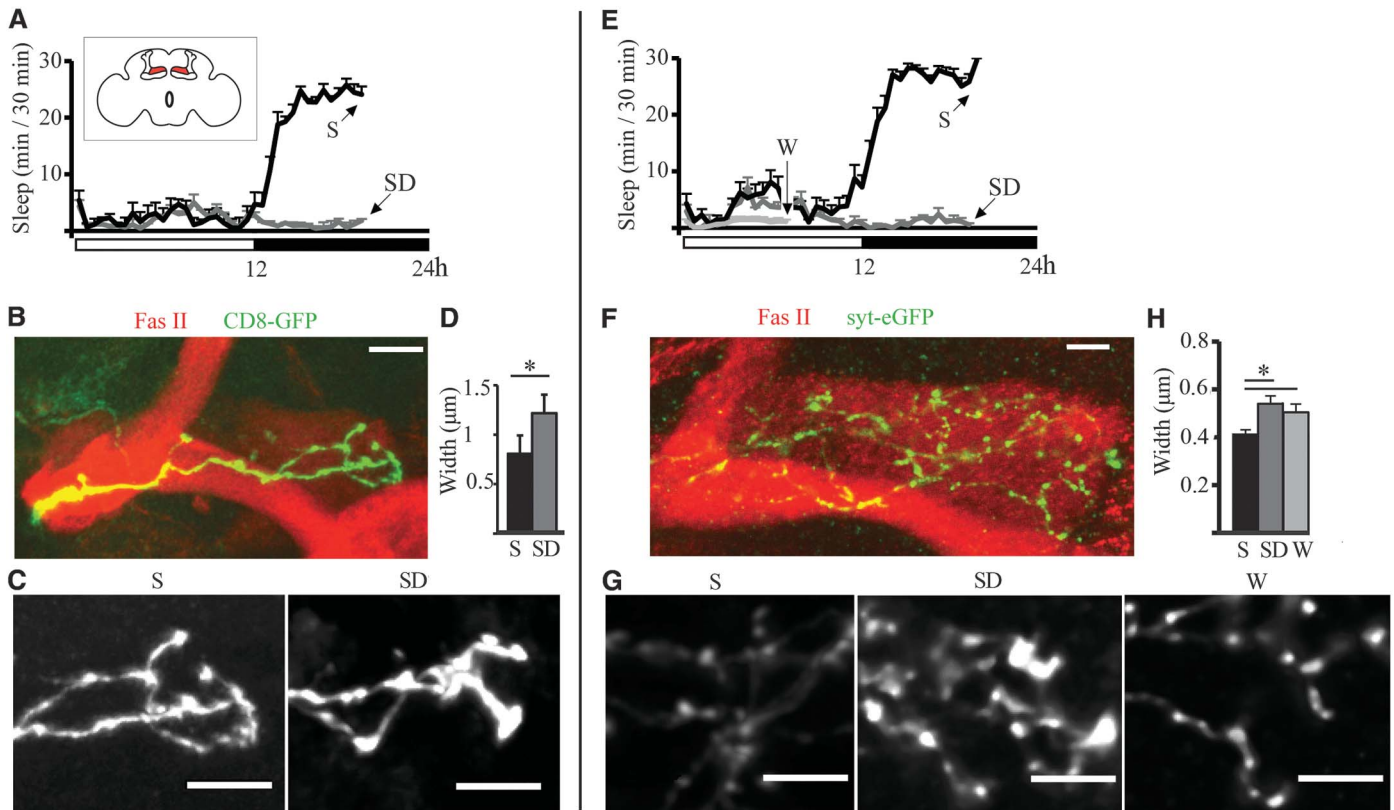


Fig. 2. Sleep/wake presynaptic changes in the gamma lobe of the mushroom bodies. (A) Mean sleep duration in female flies used for imaging after 7 hours of S or SD at night. Inset, schematic frontal section of the fly brain showing gamma lobes in red. (B) Example of MARCM clones tagged with CD8-GFP (green), which outlines gamma lobe neurons. Fasciclin II (Fas II, red) staining outlines the mushroom bodies. (C) Representative images of CD8-GFP clones from S and SD flies. (D) Mean width of axonal tips (females,

S = 26, SD = 15). (E) Mean sleep duration in flies used for imaging after W, SD, or S. (F) Representative gamma lobe with two MARCM-generated clones expressing syt-eGFP (green). (G) Representative syt-eGFP puncta from S, SD, and W flies. (H) Mean puncta width (males and females did not differ and were pooled; S = 34, SD = 26, W = 20). Mean number of tested puncta per lobe per fly was S = 28 ± 2 , SD = 29 ± 2 , W = 30 ± 2 . All scale bars, 10 μ m. All panels show mean $\pm$ SEM.

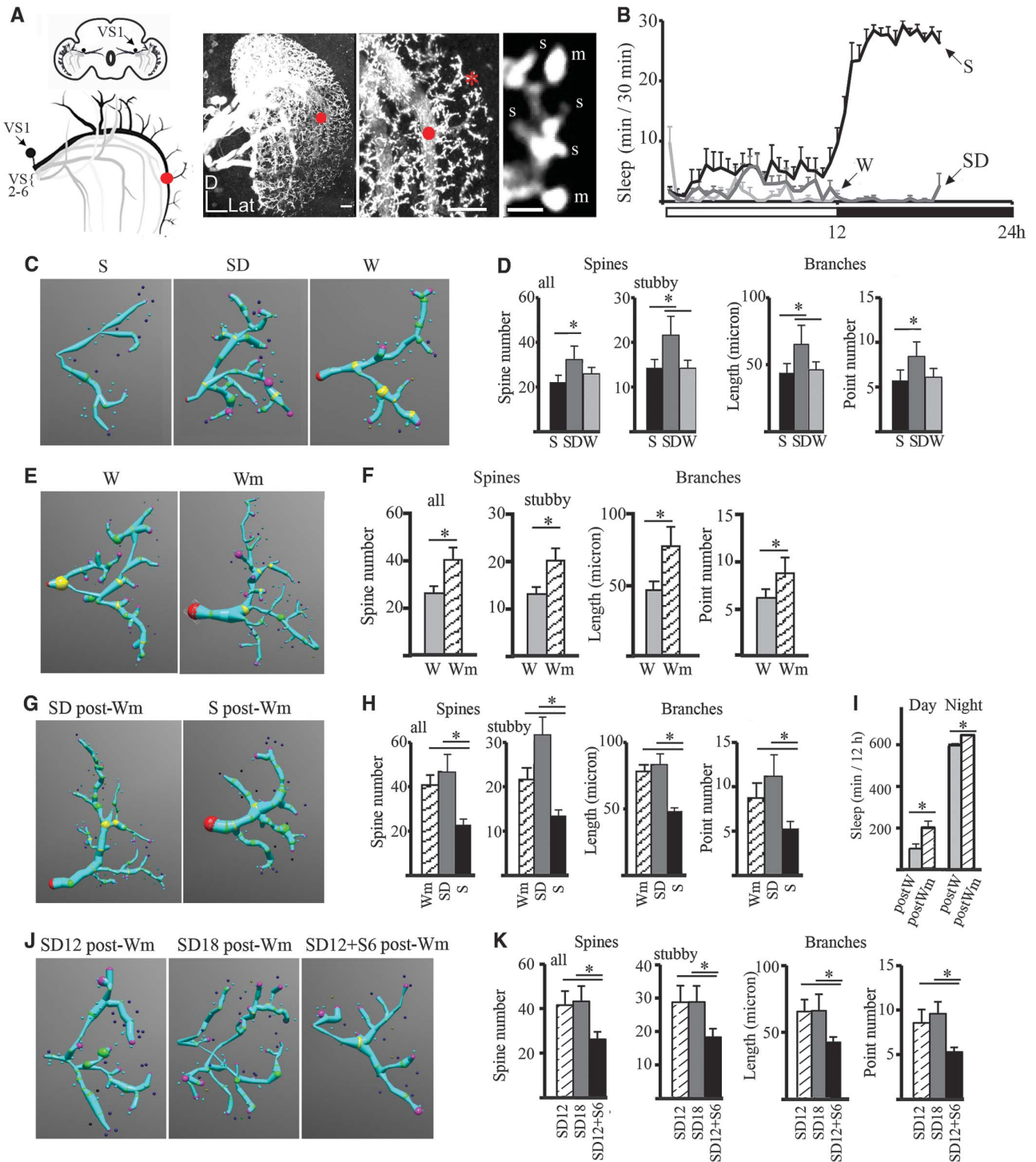


Fig. 3. Sleep/wake postsynaptic changes in VS1. (A) (Left) Frontal section of fly brain with VS neurons (branches and cell body are shown only for VS1; trunks are shown for all other VS neurons). (Right) Representative two-dimensional (2D) maximum intensity projections of 3D image stacks of VS neurons (actin-GFP driven by DB331GAL4) at low and medium resolution (left and middle scale bars, 10 μ m) and high resolution (right scale bar, 1 μ m). Red dot indicates the beginning of the scored branch. Red asterisk is above the region shown in the right panel. s, stubby; m, mushroom. (B) Mean sleep duration in females used for imaging after W, SD, or S. (C) Examples of model neurons [reconstructed using NeuroStudio (36)] from S, SD, and W flies. Model shows dendritic processes as blue cylinders connecting user-defined locations on the branch (large spheres) and spines (smaller spheres). (D) Mean number of total

and stubby spines, branch length, and branch points ($N = 10$ flies per group). (E and F) Examples of reconstructed neurons from flies awake for 12 hours in single tubes (W, $N = 10$) or in the fly mall (Wm, $N = 12$). (G and H) Examples of reconstructed neurons from flies allowed to sleep (S postWm, $N = 12$) or sleep deprived (SD postWm, $N = 11$) after 12 hours in the fly mall. [Wm = 12, same flies as in (F)]. (I) Sleep time for the 24 hours after 12 hours in the fly mall (postWm, $N = 76$). Control flies (postW, $N = 75$) spent the same 12 hours awake in single tubes. (J and K) Examples of reconstructed neurons from flies housed for 12 hours during the light period in the fly mall and then sleep deprived for 12 hours at night. Flies were then collected immediately (SD12, $N = 9$), sleep deprived for 6 hours (SD18, $N = 7$), or allowed to sleep for 6 hours (SD12 + S6, $N = 10$). All panels show mean $\pm$ SEM.

increased after sleep deprivation relative to both conditions, mainly because of an increase in stubby spines (which were the majority of scored spines) (Fig. 3D, left). The number of mushroom spines did not change ($P = 0.29$, Kruskal-Wallis test). The increase in spine number after sleep loss was associated with increased branching and lengthening of the dendritic tree (Fig. 3D), whereas spine density (number of spines divided by branch length) was similar in all conditions ($P = 0.20$, Kruskal-Wallis test). Because sleep-deprived female flies had been mostly awake during the previous light period, this suggests that these postsynaptic changes may need sustained periods of wake. Another possibility, not mutually exclusive, is that changes in VS1 spines require a wake condition richer than that experienced by flies spontaneously awake alone inside small glass tubes. Indeed, sleep-deprived flies were kept awake using vibratory stimuli, resulting in the flies often falling from the top to the bottom of the tubes. Because visually driven responses in VS neurons are stronger during flight than during nonflight (31), it is possible that these cells were activated by the fall.

To test whether a rich wake experience that engages the VS circuit is sufficient to affect VS1 synaptic morphology, we housed up to 100 flies inside a large lighted chamber ("fly mall") for an entire light period (12 hours). In the mall, flies could fly ad libitum, explore, and interact with each other. Flies were collected immediately after the mall experience and compared with flies that, as usual, had remained awake during the day in single tubes. The enriched experience in the mall

had profound morphological effects on the VS1 dendritic tree: Total branch length increased because of the addition of more branches with spines (mainly stubby), resulting in an overall increase in spine number (Fig. 3, E and F).

Once experience-dependent synaptic changes have occurred, are they stable? If not, is sleep necessary to bring synaptic morphology back to pre-enrichment levels? To answer these questions, two other groups of flies were moved back to single tubes after 12 hours of mall experience; one group was allowed to sleep for 7 hours, whereas the other was kept awake as before using mechanical stimuli. In flies that were sleep-deprived after enrichment, branch length, branch points, and spine number were at levels similar to those seen in flies collected immediately after enrichment. In contrast, in flies that were allowed to sleep after the mall experience, all morphological parameters reverted to the levels observed in awake flies kept in single tubes (Fig. 3, G and H). Moreover, spine density was negatively correlated with the amount of sleep during the last 7 hours, as well as with the maximal duration of sleep bouts (fig. S1). In another experiment, flies were housed in the mall for 12 hours during the day and then moved back to single tubes to record their sleep. During the 24 hours after the enrichment, flies slept more, both during the day and at night (Fig. 3I). Finally, in the last experiment, flies were housed in the mall for 12 hours during the day, moved back to single tubes and sleep deprived all night (12 hours), and then either collected immediately, allowed to sleep for 6 hours, or kept awake for 6 more hours. Consistent with

the previous experiments, decreases in all morphological parameters were seen only in flies that could sleep (Fig. 3, J and K), and spine density was negatively correlated with the amount of sleep during the last 6 hours, as well as with mean and maximal duration of sleep bouts (fig. S2).

Previous experiments suggest that *Fmr1* could mediate at least some of the effects of sleep/wake on synapses. *Fmr1* protein product (FMRP) is present in dendritic spines, and loss of FMRP in flies is associated with overgrown dendritic trees, larger synaptic boutons (32), and defects in developmental and activity-dependent pruning (22, 24). Notably, *Fmr1* overexpression results in the opposite phenotype, with dendritic and axonal underbranching and loss of synapse differentiation (32). Moreover, *Fmr1* expression is reduced by sensory deprivation in flies (24) and increased by sensory stimulation and enrichment in mammals (33–35).

We recently showed that FMRP levels increase in the adult fly brain during wake relative to sleep, independent of time of day or light (29), suggesting that waking experience is sufficient to affect *Fmr1* expression even after the end of development. We also showed that *Fmr1* overexpression in either the whole brain or in the mushroom bodies is associated with an ~30% decrease in sleep duration (29), and we hypothesized that this reduced need for sleep occurs because chronically high *Fmr1* levels may allow synaptic pruning to occur at all times, independent of sleep. If so, *Fmr1* overexpressing (OE) flies should fail to show increased spine density after prolonged wake. We thus overexpressed

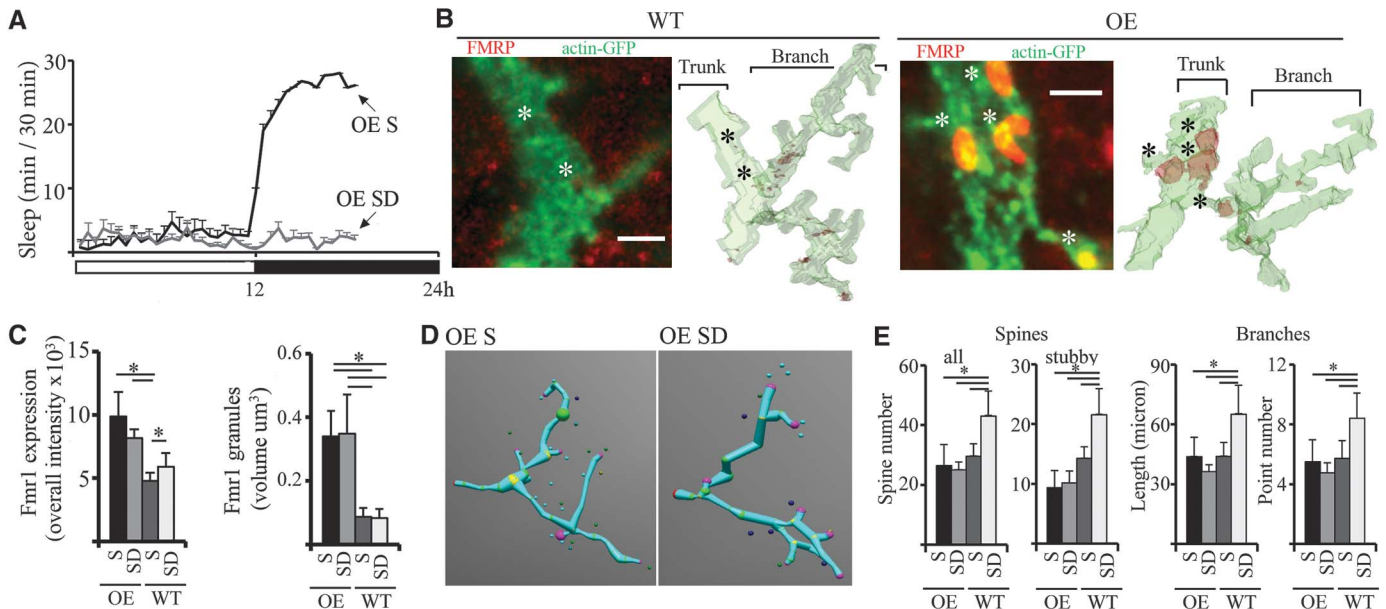


Fig. 4. Effects of *Fmr1* overexpression on synaptic complexity and sleep need. **(A)** Mean sleep duration in OE female flies used for imaging after S and SD. **(B)** (Left) Single confocal images of *Fmr1* WT and OE VS1 neurons stained for FMRP (red) and actin-GFP (green, overlap yellow). (Right) Surface plots generated by segmenting the 3D confocal stacks. FMRP localizes to granules clearly visible in OE but not in WT VS1 neurons. Scale bars, 2 μm . **(C)** Mean

overall (trunk+branch+spine regions) FMRP intensity (left), and mean granule volume (right) in WT and OE VS1 neurons. **(D)** Examples of reconstructed neurons (as in Fig. 3C). **(E)** (Left) Total and stubby spine number per branch in OE and WT harvested after S or SD. (Right) Mean branch length and number of branch points. All panels show mean $\pm$ SEM. *N* (flies): OE S = 9; OE SD = 8; WT S = 11; WT SD = 8).

Fmr1 specifically in the vertical and horizontal system of the lobula plate. OE flies were collected at night after 7 hours of either sleep or sleep deprivation (Fig. 4A) and were compared to corresponding sleeping and sleep-deprived WT controls. As expected, *Fmr1* expression was concentrated in granules along the VS1 dendritic tree (Fig. 4B), and overall *Fmr1* levels were higher in sleeping and sleep-deprived OE flies than in their corresponding controls, due to larger *Fmr1* granules in OE flies (Fig. 4C). Crucially, in contrast to WT controls, OE flies showed no increase in either spine number, branch length, or branch points after sleep deprivation relative to sleep (Fig. 4, D and E); all these parameters were similar between the two experimental groups, and their levels were close to those observed in WT flies after sleep (Fig. 4, D and E). Finally, OE flies slept less than their WT controls during baseline (-9.6% ; N of flies: WT = 110, OE = 62; $P < 0.05$, Mann-Whitney test) and showed a reduced sleep rebound after 12 hours of sleep deprivation at night (percentage of sleep recovered: OE 39%, WT = 49%; N of flies: OE = 293, WT = 420; $p < 0.05$, Mann-Whitney test; both groups lost $>90\%$ sleep during sleep deprivation). Thus, it seems that *Fmr1* overexpression was sufficient to completely abolish the wake-dependent increase in VS1 spine number, whereas the effects on sleep were small. The latter result is not surprising, because sleep need presumably results from the overall amount of synaptic plasticity occurring during wake in many brain areas, whereas *Fmr1* overexpression was restricted to a few VS neurons.

Sleep is perhaps the only major behavior still in search of a function. The results of this study support the hypothesis that plastic processes during wake lead to a net increase in synaptic strength in many brain circuits and that sleep is required for synaptic renormalization. A wake-

related increase in synapse number and strength, if unopposed, would lead to a progressive increase in energy expenditure and saturation of learning. A sleep-dependent synaptic homeostasis may explain why sleep is required to maintain cognitive performance (1). How sleep would bring about a net decrease in synaptic strength remains unknown, but in mammals, potential mechanisms favoring synaptic depression during non-rapid eye movement sleep may require the repeated sequences of depolarization/synchronous firing and hyperpolarization/silence at ~ 1 Hz observed in corticothalamic cells, as well as the low levels of neuromodulators such as noradrenaline and of plasticity-related molecules such as brain-derived neurotrophic factor (6). To what extent such mechanisms may also apply to flies remains to be determined.

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Supporting Online Material

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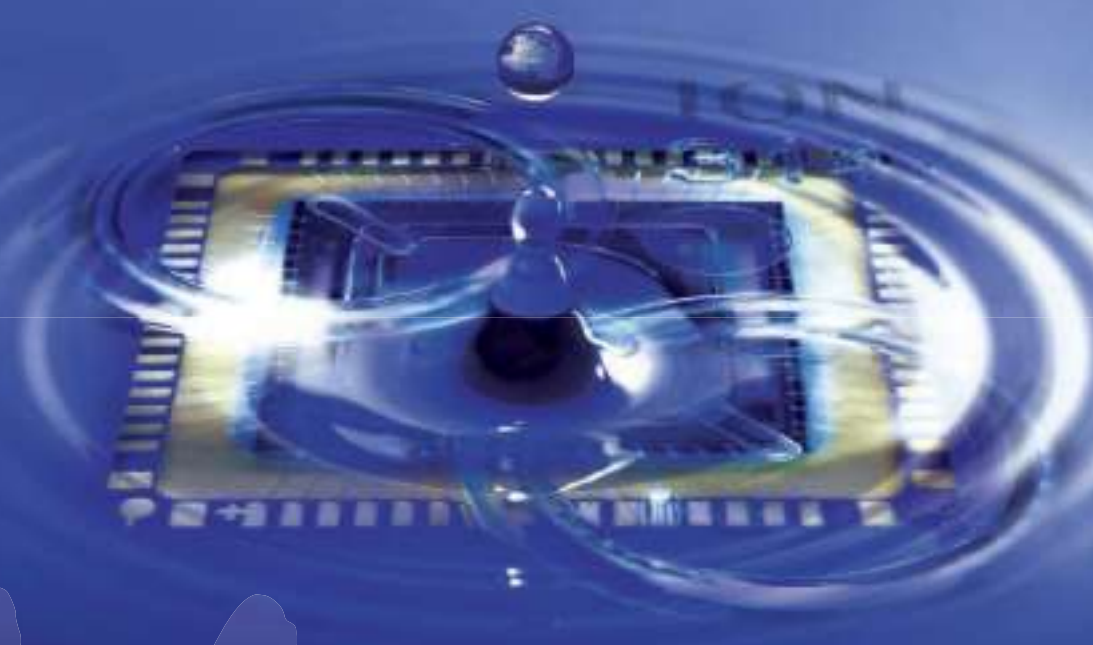
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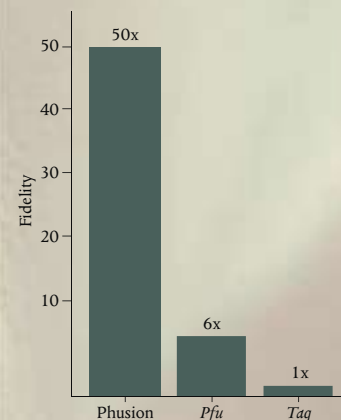
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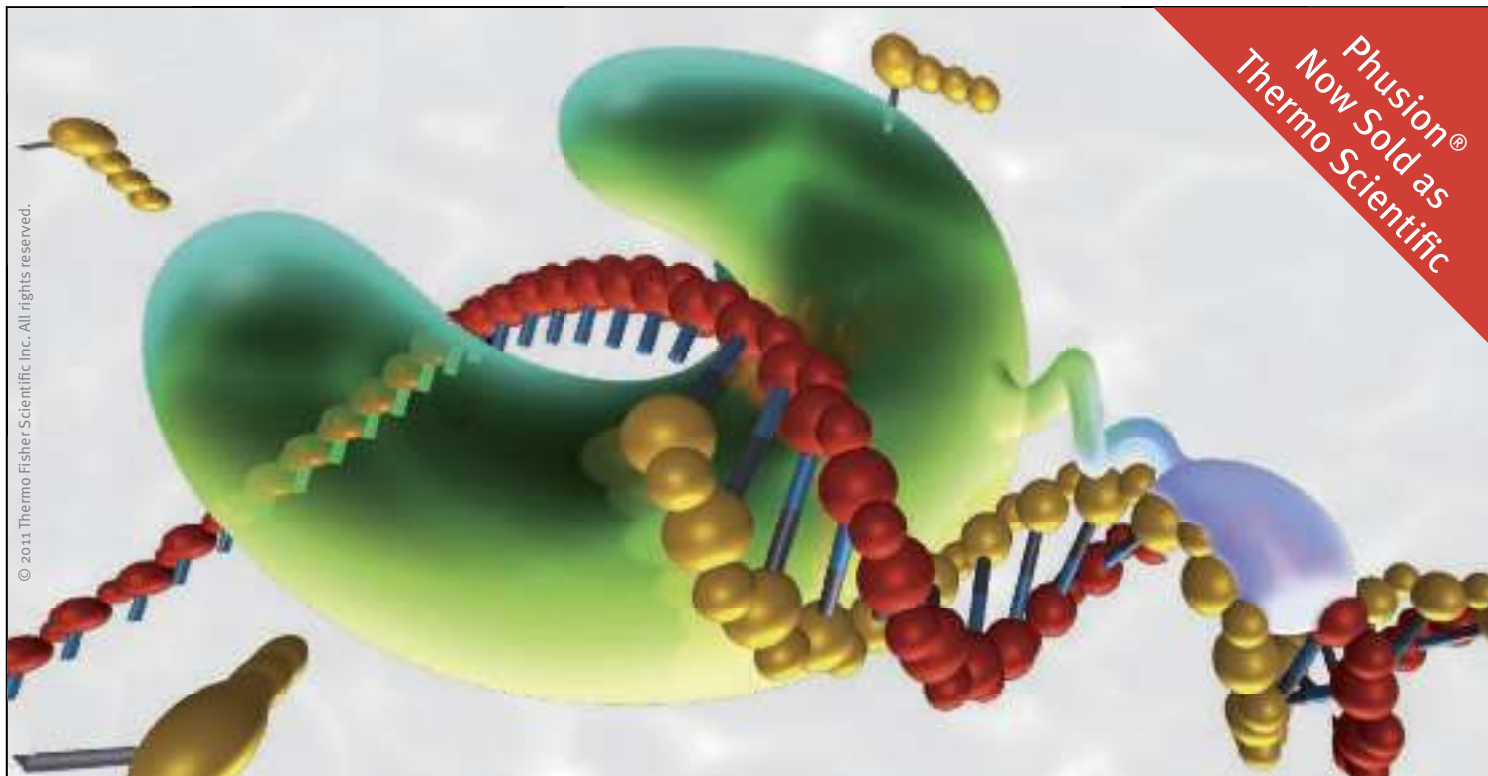


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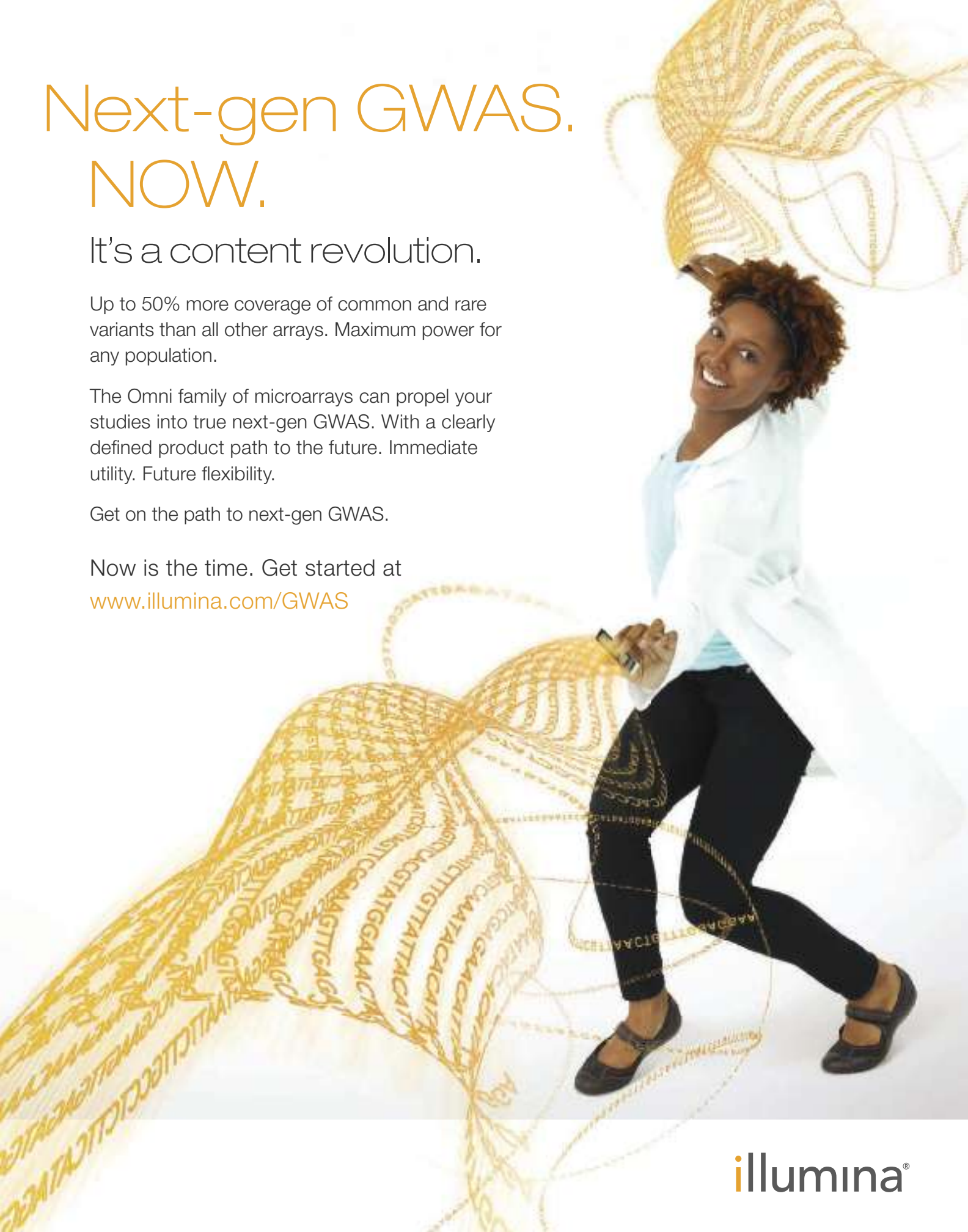
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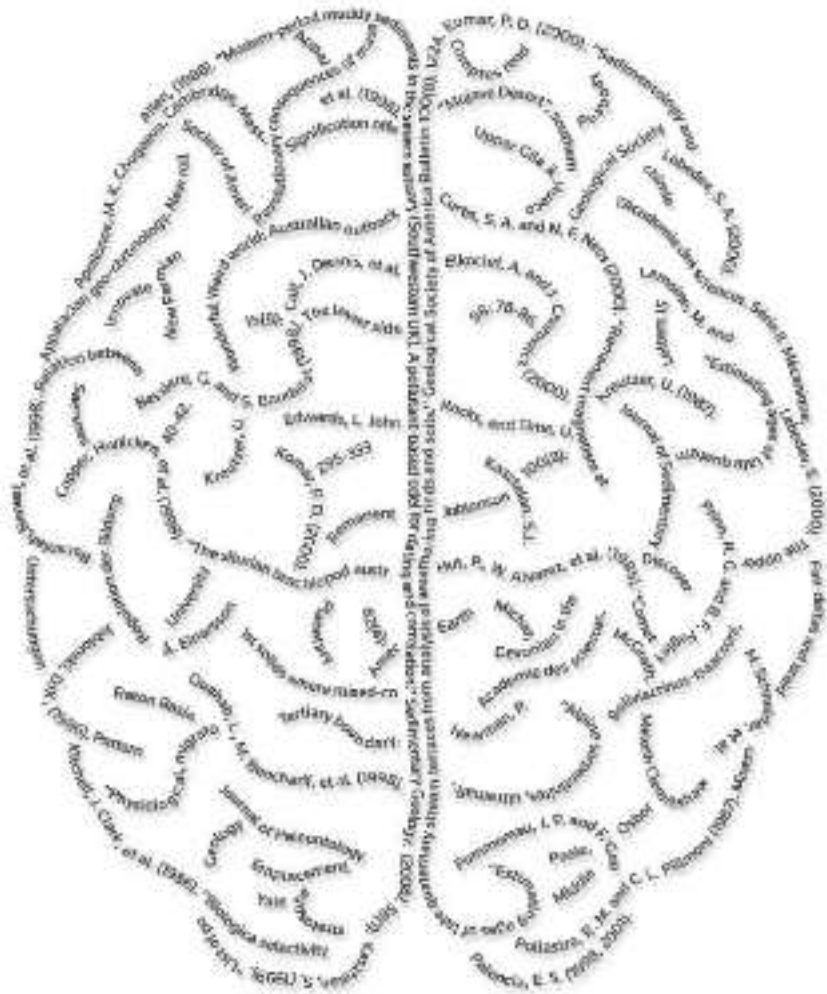
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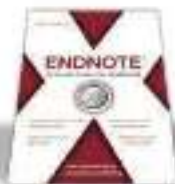
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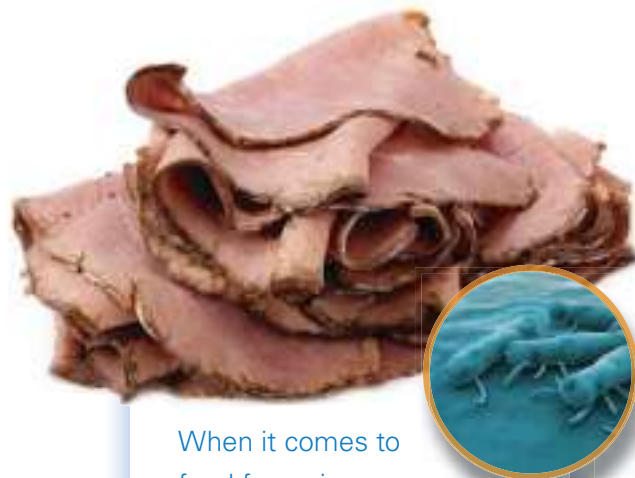
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DOES THIS TASTE FUNNY?

THE TECHNOLOGIES OF FOOD FORENSICS

It's a tough time for food manufacturers. Every week, it seems, government regulators announce that some food or other is being recalled for bacterial contamination, toxins, or carcinogens. Among the recent headlines, dozens have been sickened and two have died in an outbreak linked to contaminated pastries in Rhode Island. In January, a dioxin scare halted sales of poultry, eggs, and pork from Germany. And let's not forget radiation in Japan, oil in the Gulf, and melamine in pet food and powdered milk. If the average American never thought about food safety before, the past few years have made it abundantly clear they need to start. Fortunately, food producers and inspectors are way ahead of them. **By Jeffrey M. Perkel**



When it comes to food forensics, it's important to define an outbreak's boundaries.

According to the **U.S. Centers for Disease Control and Prevention (CDC)**, roughly 48 million Americans are sickened each year from foodborne diseases, resulting in 128,000 hospitalizations and 3,000 deaths.

Numbering among those millions were 272 individuals in 44 states and the District of Columbia who were sickened between July 2009 and April 2010. An investigation by the CDC identified "ready-to-eat Italian-style meats" as the likely culprit. Samples of deli meats from Rhode Island-based Daniele International were harboring *Salmonella*, the result of contaminated black and red pepper that the company used as a spice rub. The company ultimately recalled some 1.4 million pounds of food.

As the outbreak unfolded, the **U.S. Department of Agriculture's Food Safety and Inspection Service**, CDC, and **Food and Drug Administration (FDA)** collaborated to identify its source. Their tool of choice: Pulsed-field gel electrophoresis (PFGE) fingerprinting, the method at the heart of CDC's PulseNet tracking database.

PFGE fingerprinting uses the restriction pattern created by digesting bacterial DNA with rare-cutting enzymes as a molecular identifier of an outbreak's causative agent. According to Steve Musser, director of the Office of Regulatory Science at the FDA's Center for Food Safety and Applied Nutrition, the technique has all the advantages one would expect of a good forensics tool: It's easy, inexpensive, and widely adopted by public health laboratories. Sometimes, though, it doesn't work so well, as different (but related) bacterial strains can produce identical patterns. "Particularly with *Salmonella*, we run into serotypes that are

very difficult to resolve by PFGE," Musser says. Using this technique, "it looks like this one group of *Salmonella* are often causing the same problems."

That's a concern, Musser explains, because, when it comes to food forensics, it's important to define an outbreak's boundaries. The deli meat incident came not long after another *Salmonella* contamination event involving pistachios. The question was, were these two cases related?

To find out, Musser and his team subjected some 40 or so patient samples and food samples to whole-genome sequencing with Roche/454 Life Sciences' technology, using phylogenetics to sort the specimens into evolutionarily related clusters. The results confirmed the outbreak's association with contaminated spices (and not pistachios), while at the same time "decoupling" one apparently linked case from California. The data even hinted at the outbreak's geographical origin, as implicated strains clustered more tightly with East coast-derived samples than with West coast or international ones.

"The data are off-the-charts amazing," Musser says. And they highlight how high-end technology is empowering the food safety industry. But that doesn't mean FDA is going to start using next generation sequencing in every outbreak; it's still too expensive. "It's like a really big cannon; you only use it when you need it."

THE TECHNOLOGIES OF FOOD FORENSICS

At **SGS Consumer Testing Services**, a company that tests food on behalf of food producers, microbial testing has given way to chemical analysis, says Robert Parrish, vice president for global food. With an ever-growing roster of substances, including some 500 pesticides, prohibited globally, says Parrish, "What we have seen over the years is a much wider need for much lower level detection of things like pesticides, heavy metals, antibiotic residues, and other things that should not be in a product."

The list of technologies SGS scientists apply to assay such compounds include GC-MS and LC-MS/MS, ICP-OES (inductively coupled plasma-optical emission spectroscopy) for

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heavy metals, and in response to the radiation leak at Japan's Fukushima nuclear power plant, handheld and benchtop gamma spectrometers. "This [radiation testing] has been a big one in the past couple of weeks," Parrish says. And of course, the technologies evolve too; the company is now testing "next generation" GC/GC-TOF mass spectrometry. "The technology is not quite there to be commercialized yet," he says.

Last December, when the German poultry and pork industries were rocked by a dioxin scare, researchers at the German **Chemical and Veterinary Analytical Institute, Muensterland-Emscher-Lippe**, applied high-resolution GC-MS, says Peter Fuerst, honorary professor of food chemistry. "It provides higher separating power so you can differentiate, for instance, a mass of 321.1234 from 321.1200." Now, says Fuerst, his lab is working to determine whether GC-MS/MS, which uses mass transitions (as opposed to the mass of the parental ion) to detect ions of interest, can also satisfy European Union regulations.

Daniel Marshak, chief scientific officer at **PerkinElmer**, a company that produces analytical instrumentation for the food safety market, says his company has worked with Chinese collaborators to ensure that feed stock manufacturers don't illegally spike animal feed (which should be made with fish or plant proteins) with protein derived from ruminants such as sheep.

"Unscrupulous folks sometimes try to sneak that in small proportions into cattle feed to flesh it out, hoping we won't be able to see it by this infrared (IR) method," Marshak explains. But, using the company's IR and IR-Raman microscopes, "we can actually see which particles are from ruminant materials and which are from plant or fish material, because we get full spectral information from each of the pixels in our image." As a result, potential pathogens are excluded at the feed stock level, since "it's very difficult to do that analysis when ordering a fillet mignon," he says.

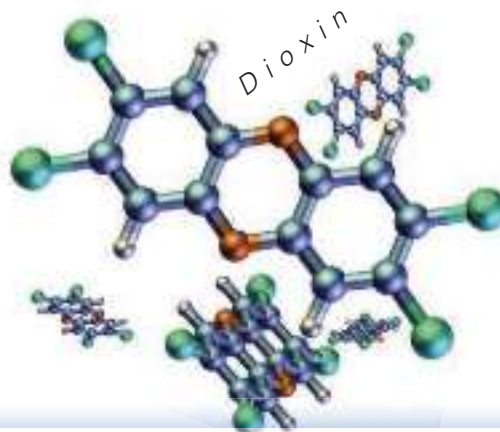
Even macroscale imaging plays a role. Japan's **Ajinomoto**, which, among other things, sells some \$100 million in frozen dumplings annually, X-rays every package for metal, rocks, and other solid contaminants, says Chief Executive Officer Takeshi Kimura. It even stores those images to better customer service. "If a customer complains about the package, we can call that image up," Kimura explains.

FOOL'S PROTEIN

Oftentimes, though, users never know food is contaminated until it's too late.

Until recently, for instance, nobody tested food for melamine because they had no reason to suspect they'd find it. But that changed in 2007, when thousands of pet owners in the United States and Canada began reporting that their cats and dogs were suddenly and mysteriously falling ill and dying of acute kidney stones.

Attention quickly focused on melamine, a chemical used as a fire-retardant and in the manufacturing of plastics, which was found in high doses in certain imported Chinese pet foods. Apparently, some vendors were spiking raw ingredients with nitrogen-rich melamine to spoof the Kjeldahl protein detection assay, which uses nitrogen as a surrogate for protein content, says Perry Martos, method development manager in the Agriculture and Food Laboratory at the **University of Guelph** in Ontario, Canada. By adding cheap melamine, these vendors could fetch



"It [high-resolution GC-MS] provides higher separating power so you can differentiate, for instance, a mass of 321.1234 from 321.1200."

a higher price for their food, as it would appear to contain more protein than it really did.

Yet as far as researchers could tell, melamine was relatively harmless, Martos says; in fact, animal studies in cats and dogs suggested they could tolerate high doses with no ill effects. But drawing on research by George Whitesides in the early 1990s, Martos realized that melamine and a related compound, cyanuric acid, could form aggregates in vivo. In the acidic stomach, the two compounds are soluble; under basic conditions such as the kidneys, they crash out of solution. "If you can imagine an instantaneous kidney stone—that's essentially the way I would perceive it," Martos said in 2007.

Martos suspects vendors could have been adding melamine for years without consequence, until cyanuric acid entered the mix as well. The question was, how to detect both compounds in a rapid, robust, and reliable way? After all, at the time nobody knew which pet food was dangerous, and which was safe.

In March 2007, he and his team developed a rapid method to resolve that question. Pet food samples were extracted, diluted—sometimes up to 10,000 times, he says—and separated on a zwitterionic-ion hydrophilic interaction liquid chromatography column coupled to a triple quadrupole MS. Total analysis time: three minutes.

"We quickly established which tissue samples were positive," he says. "And we tested the pet food that was fed to the animals that died, and there it was—the smoking gun."

Of course, pet food was only the beginning. In 2008, the scandal expanded to include human products. According to Reuters, "at least six children died and nearly 300,000 became ill from powdered milk laced with melamine."

DEEPWATER HORIZON

In the wake of the melamine crisis, and in recognition of wider emerging risks to the global food chain, **Thermo Fisher Scientific** marshaled its intellectual, financial, and analytical resources to build what it calls the Food Safety Response Center (FSRC). **continued »**

FEATURED PARTICIPANTS

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Louisiana State University
www.lsu.edu

**National Oceanic
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www.noaa.gov

PerkinElmer
www.perkinelmer.com

**SGS Consumer
Testing Services**
www.us.sgs.com

Thermo Fisher Scientific
www.thermofisher.com

University of Guelph
www.uoguelph.ca

**U.S. Centers for Disease
Control and Prevention**
www.cdc.gov

**U.S. Department of
Agriculture's Food Safety
and Inspection Services**
www.fsis.usda.gov

**U.S. Food and Drug
Administration**
www.fda.gov

Located in Dreieich, Germany, just outside Frankfurt, and representing a "very significant" investment, the FSRC boasts LC-triple quads, high-resolution mass spectrometry, a GC-triple quad, and an LC-single quad, says Gerry Broski, food safety marketing director at Thermo Fisher Scientific. "Just about every instrument you can use for analysis is in that lab." That equipment, as well as the center's three staff chemists, have one mission, Broski says: "To quickly respond in the event of a chemical contamination event and to develop a method of analysis."

Opened in April 2010, the FSRC has been activated once, Broski says, during the Gulf oil spill in July 2010. The team wanted to help develop a rapid method to detect polycyclic aromatic hydrocarbons (PAH) in Gulf seafood.

It's not as if detection methods didn't already exist. According to Edward Overton, professor emeritus of environmental sciences at **Louisiana State University**, who as part of a **National Oceanic and Atmospheric Administration** (NOAA) hazardous materials response team has worked on oil spills since the early 1980s (including the Exxon Valdez), the situation was complicated by the specific question people were asking.

Rather than asking whether the seafood was contaminated with oil in general, people wanted to know whether it was safe to eat, period. That latter question requires measuring PAHs in the low part-per-billion range, a much more rigorous test—and one, Overton notes, that is not applied to seafood caught elsewhere in the United States or that is imported.

"It's a lot easier to say [seafood] is contaminated with oil," Overton says, "you can look for hydrocarbons that are typical of petroleum. But when you have to analyze lipid-rich samples for low part-per-billion levels of polycyclic aromatic hydrocarbons, that is extremely time consuming."

The method that was applied in the summer of 2010 was a two-tiered, multiday procedure, Overton says. The first step was a so-called "organoleptic" analysis: Experts "sniffed" the food,

searching for a sulfurous whiff of *eau-d'-petroleum*. Samples that passed that test were then subjected to a time-consuming GC-MS assay. Tissue samples were homogenized into a "milkshake," and treated with base to crack open the cells. PAH resides in lipids, so the next step was to extract the cellular soup three or four times with organic solvents, concentrate the resulting material, and inject it onto the GC-MS.

"It's a long, laborious, pain-in-the-butt process," Overton concedes.

Faster methods have since been devised. FDA developed one two-day procedure based on LC-fluorescence spectroscopy, for instance, while chemists at Thermo's FSRC used GC-MS/MS to get answers in just three and a half hours. According to Broski, the trick isn't developing a working protocol per se, but rather one that is straightforward, reliable, and reproducible. "A method has to be robust enough that it can be replicated in multiple labs," he explains. "Sample preparation accounts for a substantial portion of the workflow, and we have designed our methods to eliminate as much variability as possible."

NO MORE BAD EGGS?

Motivated in part by the spate of food recalls and outbreaks of the past few years, the U.S. Congress in 2010 passed the FDA Food Safety Modernization Act, which one report called "the largest overhaul of U.S. food safety laws since 1938." The Act, according to the FDA, "aims to ensure the U.S. food supply is safe by shifting the focus of federal regulators from responding to contamination to preventing it." This "will help bring the United States into the 21st century," says Parrish.

Yet despite the best intentions of government and industry, with so many players, and with an intricately interconnected global food chain, the food we eat can never be completely safe. In any given meal, Marshak says, an individual might consume shrimp from Southeast Asia, wine from South Africa, grain from the United States, and fruits and vegetables from Europe, Latin America, and Australia. "The food that passes your lips, both in processed or raw or cooked form, comes from all over the world," Marshak says.

Those countries have different customs, standards, and regulations, not all of which necessarily rise to the levels expected by U.S. consumers. Producers looking to game the system (clenbuterol-laced pork, anyone?) don't help. But even were that not the case, for today's food inspectors the goal line keeps moving.

For instance, says Paul Zavitsanos, worldwide food industry marketing director at **Agilent Technologies**, variations in temperature profiles in the Gulf Stream are causing increased algal blooms over mussel beds. Some produce paralytic shellfish toxins, Zavitsanos says, so there's a need for methods to detect them. Next week, though, the threat could be different. "It's a constant game of catch-up," he says.

"For many, it's quite scary," concedes Parrish, "but what will you do? Become a vegetarian, you could be eating too many pesticides. If you eat too much fish, it could be mercury or antibiotics. So eat everything in moderation and enjoy yourself."

Jeffrey M. Perkel is a freelance science writer based in Pocatello, Idaho.

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(McGraw-Hill Dictionary of Scientific and Technical Terms, 4th Edition).

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Reply by: Applications will be reviewed starting August 15, 2011 and will be accepted until this position is filled.

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Further details for all positions may be found at www.ecohealthalliance.org/about/careers. All positions are based in New York and require some international travel. Review of applications will begin August 1 and continue until positions are filled. Candidates should submit a CV, 2-page cover letter stating clearly the position of interest and career goals, and e-mail addresses for two references to jobs@ecohealthalliance.org. Please include the position title in the subject of the e-mail.

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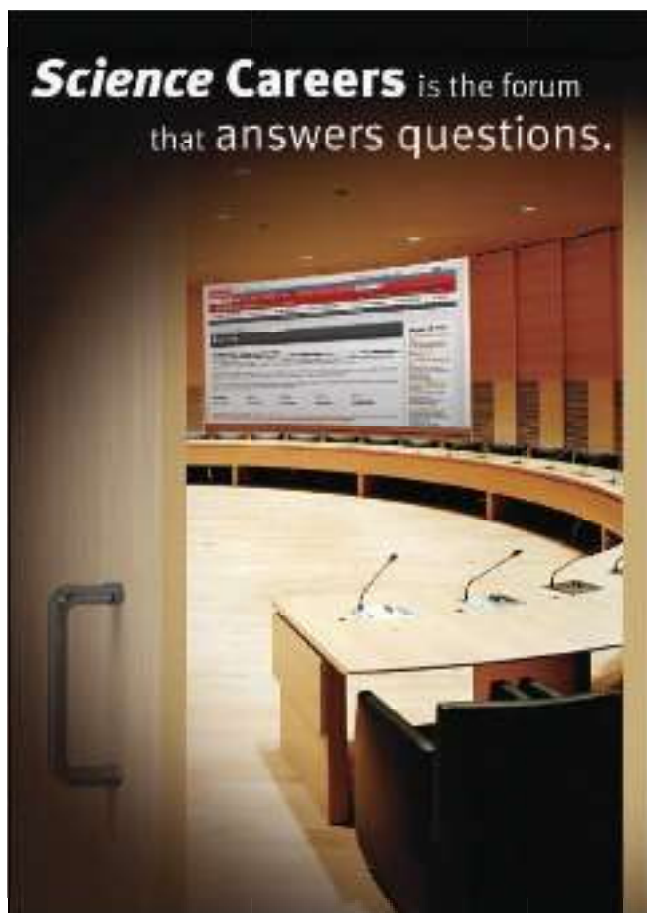
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The Center for Biomedical Imaging animal imaging facilities are located in a new Bioengineering Building, adjacent to a new Drug Discovery Building, and along with ample laboratory/office space, houses a Bruker 7T/300 MRI, a Caliper Life Sciences IVIS 200 Bioluminescent instrument and a Siemens Inveon micro-CT/PET. Adjacent to this site is the human imaging facility that houses a Siemens TIM Trio 3T MRI system, 100% dedicated to research. Several clinical Siemens 1.5 and 3T MRI systems are also available for research.

The Hollings Cancer Center is a National Cancer Institute designated cancer center. MUSC also holds an NIH-funded Clinical and Translational Science Award and there are multiple complementary shared resources in place, including a Drug Discovery Screening Core, a Drug Metabolism and Clinical Pharmacology Core, and a Proteomics facility.

Located on the Atlantic coast of South Carolina, Charleston boasts one of the nation's most historic and picturesque downtown areas, beautiful beaches, and international cultural events such as the Spoleto Festival USA.

Interested candidates should send (via e-mail) a statement of research plans and career objectives along with a CV and contact information for three references to: **Dr. Joseph A. Helpert, Vice Chairman for Radiology Research, South Carolina CoEE Endowed Chair in Brain Imaging, Director, Center for Biomedical Imaging; radresearch@musc.edu.**

Application Deadline: Until filled

MEDICAL SCHOOL FACULTY

St. Kitts Medical Campus

The University of Medicine and Health Sciences is a premier, for-profit boutique off shore medical school located on the exotic Caribbean Island of St. Kitts. Our ocean front state of the art campus is comparable to the best medical schools in the United States. We maintain small classes and provide a personalized education catering to the individual needs of the students. To date we have invested over 50 million dollars into our campus facilities and infrastructure. we are seeking the following full time faculty:

MEDICAL MICROBIOLOGY BIOCHEMISTRY PHYSIOLOGY

Preference will be given to candidates who have a minimum of 4 years experience teaching in a U.S. or Canadian medical school. Candidates must have a minimum of an earned PhD in their discipline. Candidates additionally holding an M.D. degree will be given preference. Salaries and benefits are competitive and commensurate with experience. A significant portion of the salary is offered tax free.

Candidates should forward their CV's
and cover letter to: hr@umhs-sk.net

For further details about

The University of Medicine and Health Sciences

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The Institute for Life Sciences (IfLS) was recently founded with substantial investment by the University of Southampton. As part of that investment we seek five Senior Research Fellows. The IfLS is the University's focus of activities spanning Biomedicine, Bioscience, Bioengineering and related teaching. Fellows will be recruited in the theme areas below. You will be expected to work with multidisciplinary collaborative groups in a leadership role, developing self-funded and independent research programmes. You will be offered a five year contract with the opportunity for tenure in the appropriate faculty.
www.southampton.ac.uk/ifls

- **Applied Neuroscience** - An experimental neuroscientist with an interest in *in utero* and/or postnatal central nervous system development
- **Biofilms and Microbial Communities** - Incorporate ecological or evolutionary approaches to the understanding of biofilms and microbial communities
- **Ecosystem Services** - Bridge the disciplines of complexity science and ecosystem services science through ecological/environmental approaches
- **Imaging and the Nanodomain** - Develop advanced optical imaging techniques for the study of biological systems
- **Hybrid Biodevices** - Work at the interface between engineering and the life sciences in the sensor technologies and diagnostics
- **Translational Immunology** - Join on-going research with physical sciences, electronics, engineering, mathematics and computing

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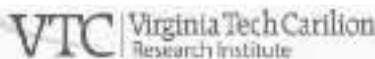


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Faculty Position in Tumors and Cancer of the Nervous System

The Virginia Tech Carilion Research Institute (VTCRI) in Roanoke, Virginia (<http://research.vtc.vt.edu/>) is recruiting a faculty member working in the area of nervous system tumor biology and/or malignancies, throughout the lifespan. The position may be filled at the Assistant, Associate or full Professor level. The successful candidate will have a Ph.D. (or M.D./Ph.D. or D.V.M./Ph.D.) and postdoctoral training experience. Established investigators with strong innovative research programs and extramural funding are encouraged to apply although promising junior candidates will be considered. Start-up packages, facilities and support are highly competitive. The VTCRI that opened in the summer of 2010 and with 12 research teams in structural biology, molecular and cellular neurobiology, cognitive neuroscience and behavior is in the process of recruiting an additional 15-20 research team leaders. The Institute has state-of-the-art facilities in optical, high field electron and magnetic resonance imaging, as well as structural biology, molecular biology, electrophysiology, computational and high capacity data analysis/storage and rodent/human behavior facilities. During this period of major growth of the new institute, we are especially interested in colleagues who enjoy a highly collaborative environment and interacting with investigators from their own, as well as other disciplines including those working at molecular, bioengineering, computational and behavioral levels with animal models of human disease and/or humans. Investigators using molecular genetic approaches to develop innovative approaches for identifying biomarkers, novel diagnostics and potential therapeutic targets are encouraged to apply. The VTCRI is immediately adjacent to the Carilion clinic and hospital and the new VTC School of Medicine where all medical students carry out research projects. The Institute has strong collaborative ties with the Virginia Bioinformatics Institute and the School of Biomedical Engineering and Sciences at Virginia Tech (VT), as well as with the VT Departments of Biological Sciences, Biochemistry, Physics, Psychology and the College of Veterinary Medicine. The research institute and medical school are located in the picturesque Roanoke Valley midway between Washington, DC and Charlotte, NC.

Interested and competitive candidates should send a cover letter, their full CV, statement of research plans, and the names, full addresses and email addresses of three references to the attention of: NSC position-VTCRI at secastle@vtc.vt.edu before July 25, 2011, as well as posting these materials on the Virginia Tech jobsite at <http://www.hr.vt.edu/employment/> under position #0110562. The three support letters should also be sent by email directly from the referees to Sarah Castle at secastle@vtc.vt.edu indicating NSC Position.

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Full-Time Clinical Faculty Position in Veterinary Microbiology

This unique faculty position provides an opportunity for an outstanding clinical veterinary microbiologist to join a team of interdisciplinary faculty dedicated to improving animal and human health globally. The successful applicant will serve as the Section Head for the Washington Animal Disease Diagnostic Laboratory (WADDL www.vetmed.wsu.edu/depts_waddl) Bacteriology/Parasitology and Central Processing sections, and will serve as Director of the Clinical Microbiology Training Program. In addition, the clinical microbiologist will hold an academic appointment in the Paul G. Allen School for Global Animal Health. Founded by gifts of the Bill and Melinda Gates Foundation and Paul G. Allen Family Foundation, the mission of the Allen School is to improve human health by control of infectious diseases at the animal-human interface (<http://www.globalhealth.wsu.edu>). The clinical microbiologist will be expected to work closely with faculty to further develop the Allen School's microbiology training program and to demonstrate a commitment to the School's public health and humanitarian mission as a valued team member in extramurally funded research and development projects. Research and graduate education in the Allen School is focused on infectious diseases, including both zoonotic pathogens and pathogens that constrain livestock development in resource-poor countries. In addition to current modern research facilities, the Allen School is presently constructing a new BSL2/3 research building.

A doctoral degree (DVM or equivalent, or PhD) and post-DVM or post-PhD clinical microbiology or research experience is required. A DVM and PhD degree is preferred. Other preferred qualifications include diplomate status in the American College of Veterinary Microbiologists (or intention to obtain ACVM certification), experience in delivery of clinical bacteriology services in an accredited animal diagnostic laboratory, a record of training DVM residents in clinical microbiology, and a demonstrated ability to support high impact research projects through collaboration. Applicants with a record of, or strong potential for, publication in high quality international journals, and research relevant to the mission of the Allen School are encouraged to apply. The appointment may be as a Clinical Assistant or Clinical Associate Professor, depending on experience and record of achievement.

For further information about the position, please see the Notice of Vacancy at <http://www.vetmed.wsu.edu/employment/>. Candidates are encouraged to submit an early application. Review of applications will begin on **August 15, 2011**. To apply for this position, please visit WSUJobs.com; you are required to submit a letter of application, curriculum vitae, and names and addresses of three references. For more information or if you have questions please contact **Ms. Jill Griffin, Paul G. Allen School for Global Animal Health, Washington State University**, at griffinj@vetmed.wsu.edu or 509-335-5861.

WSU is an EEO/AA Educator and Employer. Protected group members are encouraged to apply.



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Eligibility

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- Candidates must have been awarded a postdoctoral position in the U.S. host research institution.

Details regarding the fellowship are available at www.eglc.org

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Research Co-ordinator

£36,862 - £46,696 pa

A 5-year Research Co-ordinator position is available in the group of Professor M J Rosseinsky. A PhD in Chemistry, Physics or Materials Science, excellent research skills in synthetic materials chemistry (oxides, porous materials, hybrid nanomaterials) plus an excellent publication record and demonstrated ability to take responsibility in research organisation are essential. The role will focus on the most challenging areas of new materials synthesis and characterisation, and has considerable scope for contribution to strategic planning and development of new research directions. The role is ideal for ambitious candidates with high levels of achievement who have the potential to become future leaders in materials science.

Job Ref: R- 572400/S

Closing Date: 22 July 2011

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COOPERATIVE EXTENSION SPECIALIST/AGRONOMIST SUBTROPICAL HORTICULTURE

The Department of Botany and Plant Sciences invites applications for an Assistant Cooperative Extension Specialist/Assistant Agronomist (CE 75%/AES 25%, 11-month, tenure track) in Subtropical Horticulture. The position will sustain the subtropical tree crop industries of California, for example, by improving water, nutrient and salinity management while increasing yield efficiency of commercially valuable fruit and grower net return. The appointee will develop a high impact, independent and nationally competitive research program, obtain extramural grant funds, and establish a strong extension education program. The position will be available July 1, 2011. Applicants must hold a Ph.D. in Horticulture, Plant Physiology or related fields with a minimum of 1-3 years postdoctoral experience.

Evaluation of applications will begin **September 1, 2011**, and continue until the position is filled. Interested individuals should submit (1) a curriculum vitae, (2) a statement of research and extension interests, and (3) have three letters of recommendation sent to: **Chair, Cooperative Extension Specialist Search Committee, c/o Department of Botany and Plant Sciences, 2118 Batchelor Hall, University of California, Riverside, CA 92521-0124; E-mail: bpsrecruit@ucr.edu; FAX: (951) 827-4437.** For additional information on the Department and the campus visit <http://cnas.ucr.edu/> and <http://www.plantbiology.ucr.edu/>.

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Cancer Institute

NYU LANGONE MEDICAL CENTER

Program Leader and Scientist, Neuro-Oncology Research Program

The NYU Cancer Institute

The New York University Cancer Institute (NYUCI) at the NYU Langone Medical Center is seeking a basic scientist (PhD, MD, MD/PhD) to lead a neuro-oncology research program that will further catalyze effective interactions among our existing community of laboratory and clinical investigators. The candidate's work should be focused on discovering fundamental pathways that drive the initiation and progression of both benign and malignant human brain cancers in children and adults and translating these discoveries into prevention and treatment strategies. Competitive candidates should have a strong record of scientific achievement and a sustained history of extramural funding. A strong, multi-disciplinary clinical neuro-oncology program with high accrual of pediatric and adult patients, a funded and extensive brain tumor bank, and an experienced clinical research staff engaged in investigator-initiated clinical trials will provide abundant opportunities to pursue novel clinical and translational research ideas. Newly developed, multidisciplinary programs in Neurofibromatosis (NF1 and NF2) provide further opportunities for scientific collaboration. The NYUCI is an NCI-designated cancer center with over 200 members who are engaged in discovering the origins of human cancer, with the goal of eradicating the personal and societal burden in our community. Comprehensive shared resource facilities at NYU, including genomics, proteomics, tumor banking and clinical trials facilitate scientific discovery and clinical application. This position will include an appointment in the rapidly expanding program in neurosciences, a joint venture between the NYU main campus and the School of Medicine, which has culminated in establishment of the new NYU Neurosciences Institute. NYU Langone Medical Center, a world-class patient-centered integrated academic medical center, is one of the nation's premier centers for excellence in healthcare, biomedical research, and medical education.

Prospective candidates should forward their curriculum vitae and letter of interest to **Robert J. Schneider, Associate Director, NYU Cancer Institute, c/o Margarita Nunez, NYU Cancer Institute, 215 Lexington Avenue, 15th Floor, Suite 1510, New York, NY 10016 or email Margarita Nunez at margarita.nunez@nyumc.org.** We are an equal opportunity employer fostering diversity in the workplace.

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POSITIONS OPEN

FACULTY POSITIONS

**University of Pittsburgh Cancer Institute and
Department of Pharmacology and
Chemical Biology**

Applications are invited for a tenure-stream **ASSISTANT PROFESSOR** level faculty position at the University of Pittsburgh Cancer Institute (UPCI). The incumbent will have primary appointment in the Department of Pharmacology, University of Pittsburgh. We are particularly interested in the recruitment of a Ph.D. scientist working in the area of nuclear hormone receptor signaling in lung cancer prevention and/or treatment.

Successful candidates should have a track record of extramurally supported research grant funding, a strong publication record, and excellent communications skills. Salary and benefits will be commensurate with experience.

Applicants should provide a one-page statement of research objectives, curriculum vitae, and contact information for three professional references by August 15, 2011 to: c/o Vijaya C. Gandhi, Ph.D., MBA, Associate Director for Administration and Strategic Planning, UPCI, 5150 Centre Avenue, Suite 532, Pittsburgh, PA 15232, E-mail: gandhivc@upmc.edu.

The University of Pittsburgh is an Affirmative Action/Equal Opportunity Employer.

ASSISTANT PROFESSOR, Microbiology, Nine-month, tenure-track. The Department of Biology, The University of Texas at Tyler, invites applications for a nine-month, tenure track, Assistant Professor position. The successful applicant will establish an extramurally funded research program in environmental microbiology, evolutionary microbiology, or microbial genomics to complement existing departmental strengths in ecology and evolution and teach courses in microbiology. Qualifications: Ph.D. in microbiology, productive postdoctoral experience, strong publication record, grantsmanship, and teaching experience. Applications must include (as a single PDF file) curriculum vitae, statements of research interests and teaching philosophy, and reprints of three publications sent to **Dr. James Koukl**, chair, Microbiology Search Committee at e-mail: jkoukl@uttyler.edu. Three letters of reference should also be sent to the same address. Full position description found at website: <http://www.uttyler.edu/biology>. This is a security sensitive position and subject to criminal history check. The University of Texas at Tyler is an Equal Opportunity Employer; women and minorities are encouraged to apply.

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